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QUALITY OF SOME ESSENTIAL OILS FROM HERBS AND SPICES GROWN IN ALBERTA

by

© MAMAT BIN EMBONG

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF FOOD SCIENCE

EDMONTON, ALBERTA

SPRING, 1974

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled QUALITY OF SOME ESSENTIAL OILS FROM HERBS AND SPICES GROWN IN ALBERTA submitted by Mamat Bin Embong in partial fulfilment of the requirements for the degree of Master of Science.

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DATE December 10/1973..

ABSTRACT

For the past three years the Department of Food Science at the University of Alberta and the Horticultural Research Centre at Brooks, Alberta, have been studying the feasibility of growing spices and herbs in Alberta on a commercial scale. This study reports the composition and quality of the essential oils for the crop years 1970 through 1972 from the seeds of anise (Pimpinella anisum L.), caraway (Carum carvi L.), dill (Anethum graveolens L.), fennel (Feoniculum vulgare Mill.), herbs of peppermint (Mentha piperita L.) and sage (Salvia officinalis L.). The oils were obtained by steam distillation of dried herbs and seeds.

The analyses of the essential oils were carried out by means of thin-layer and gas-liquid chromatography and infrared and mass spectroscopy. Thin-layer chromatograms of anise oil revealed 13 spots. The two major spots corresponded to hydrocarbons and to benzene related constituents. There were seven spots on thin-layer chromatograms of caraway oil. The spots corresponding to hydrocarbons and carvone were the two major ones. Thin-layer chromatograms of dill oil showed eight spots, of which the spots for hydrocarbons, carvone and an alcohol with linalool and 1,8-cineol were the three major ones. There were ten spots on chromatograms of fennel oil. The major spots were those of benzene related constituents and hydrocarbons. Thin-layer chromatograms of peppermint oil revealed eleven spots, of which spots for hydrocarbons, menthyl acetate, menthone and menthol were the major ones. Sage oil showed nine spots on thin-layer chromatograms. The spots for borneol, linalool, 1,8-cineol, camphor, thujone, bornyl acetate and hydrocarbons were the major ones.

The gas-liquid chromatograms of the essential oils studied revealed major, intermediate, minor and trace constituents. Anise oil contained fifty-four constituents with 57.42 to 75.21% of trans-anethole as the major one. Oil of caraway had a total of forty-seven constituents, of which carvone (38.79%) and limonene (48.77%) were the major ones. Dill oil contained a total of thirty-eight constituents, of which carvone (43.26 to 48.46%) and limonene (33.11 to 40.82%) were the major ones. Fennel oil had a total of fifty constituents, of which trans-anethole (39.19 to 68.90%), limonene (8.29 to 19.78%) and fenchone (10.83 to 16.12%) were the major ones. Peppermint oil contained a total of fifty-five constituents, of which menthol (32.33 to 44.18%) and menthone (32.62 to 21.24%) were the major ones. Sage oil had a total of sixty constituents, of which thujone (23.70 to 29.06%), humulene (13.12 to 16.44%), camphor (8.34 to 10.31%) and β-caryophyllene (7.67 to 9.34%) were the major ones.

The quality of anise seed oil from Brooks was better than the oil from the whole herb and was better than commercial oil obtained from Michig . Although the quality of caraway oil from Brooks was slightly lower than the commercial oil from Michigan, an improvement could be achieved by distilling the oil from more matured seed. The quality of dill seed oil from Brooks, was comparable to the oils from the U.S.A. and Europe. The bitter fennel seed oil from Brooks, 1972 crop, due to the high content of trans-anethole was of better quality than that of the 1971 crop which was distilled from the whole herb. In addition the quality of the oil was comparable to that of oils from the U.S.A. and Europe. The quality of peppermint oil from Brooks, depending on the stage of growth, is comparable to that of commercial oil from

Michigan. The quality of sage oil from the Brooks, 1971 crop was better than that of the 1972 crop and commercial oil from Michigan.

The yield of oil from spices and herbs grown under climatic conditions of Alberta appeared to be dependent on the crop year, total hours of sunshine and the length of the frost-free period. However, the quality of the oils was still comparable to the oils commercially available on the world market.

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INTRODUCTION

The food industry is constantly seeking new methods of improving or redesigning its products for both gustatory and financial reasons. This has created a lucrative market for spices, oleoresins and essential oils for domestic use by producers of soft drinks, baked goods, desserts, confections, meat and fish products, and alcoholic beverages. Currently, all of these flavoring products are imported.

For the past five years a team at the University of Saskatchewan has been studying the feasibility of growing the peppermint plant as a cash crop for farmers. A few years ago the Canadian Department of Agriculture Research Branch at Morden, Manitoba, and the Provincial Horticultural Research Centre at Brooks, Alberta, began an investigation to determine whether peppermint and other herbs and spices can be grown successfully under our climatic and soil conditions.

This complex investigation involves the quality, horticultural, agrological, economic and marketing aspects. This study reports the chemically assessed quality of essential oils from some herbs and spices grown in Alberta and their comparison with similar oils supplied by the world market.

II. REVIEW OF LITERATURE

A. Anise Seed Oil (Pimpinella anisum L.)

Anise seed and oil are characterized by a very strong, licorice-like flavor and odor. Anise oil, rather than the licorice root itself, is generally used to provide licorice flavoring. Its taste is sweet and aromatic. Anise oil is widely used as a flavoring material in liquorice, toothpaste, baked goods, carbonated drinks, confections and alcoholic beverages. It is the basis of the French cordial "anisette", the popular Turkish alcoholic beverage "raki" and Bulgarian and Greek "mastika". Anise-flavored aguardiente (distilled from sugar cane) is one of the most universally popular alcoholic drinks throughout Latin America. Other products which have anise oil or anethole as a flavoring ingredient are canned foods, pickles and spice blends. The flexible concentration level of anise oil makes it an ideal flavor base. The FDA "white list" permits the use of anise oil in a concentration up to 3500 ppm. Anise oil is utilized in medicine for its carminative and expectorant properties and as a masking agent for flavoring bitter-tasting drugs. It has been reported that in flavoring 100 lb of the best grade of dry anise seed is equivalent to 3 lb of oil ("the spice equivalent").

There are two botanically different plants whose fruits yield essential oils of similar color, flavor and, hence, chemical composition. Star anise oil is obtained from Illicium verum Hooker (family Magnoliaceae) and the other oil, which is true anise oil and has finer and delicate flavor, is distilled from anise fruit, Pimpinella anisum L. (family Umbelliferae). This true anise is an annual plant indigenous to Levant

but it is widely grown in Europe, North Africa, North America, China and Chile (Guenther, 1950; Anonymous, 1970a).

When the seed is near maturity, alternate rainy and dry periods cause the seed to turn brown, thus greatly impairing the quality (Guenther, 1950). Some agrological requirements for growing this plant are given in Appendix A-1.

According to Gildemeister and Hoffman (1931) the oil yield of anise seed of various origin ranged from 1.5 to 6.0%. Fisher et al. (1945) reported that the oil yield of commercial anise seed from various origins varied from 1.9 to 3.1% with an average of 2.29%. It has been claimed (Anonymous, 1970a) that anise seed yielded from 1.5 to 3.5% volatile oil. It was observed by Chernukhin (1929) that 5% more oil could be distilled from ground seed than from whole seed.

The volatile oil contains primarily anethole (80-90%) along with methyl chavicol (an isomer of anethole) and p-methoxyphenylacetone (anise ketone) (Guenther, 1950). Using gas-liquid chromatography, El-Deed et al. (1962a) found that the chromatogram of anise oil had four peaks, two of which were identified as anethole (88.61%) and anisaldehyde (3.01%). In the study of the quality of some umbelliferous essential oil plants Tsvetkov (1970) found that the yields of oil distilled from central, first order and second order umbels were 0.34, 0.35 and 0.25%, respectively, and their respective percentage compositions were estragole 8.64, 7.51 and 7.71; anise ketone 0.88, 0.41 and 0.85; and anethole 87.30, 89.50 and 88.40. From this study he concluded that a high yield of fine quality oil could be obtained when the seeds in the central umbels were fully ripe.

In characterization of essential oils by thin-layer chromatograph (TLC) using 10 to 15% ethyl acetate in hexane as a solvent system Reitsema (1954) found that anise oil produced five spots with Rf values of 0.02, 0.18, 0.28, 0.62 and 0.94. Zacsko-Szász and Szász (1965) analyzed anise oil by using a mixture of benzene:chloroform (1:1) as a solvent and reported the Rf values as follow: anisic acid 0.0 - 0.06, methyl chavicol 0.28, anise ketone 0.33, anisaldehyde 0.44 and trans-anethole 0.83.

The quality of anise oil depends mainly on the amount of trans-anethole, the higher the amount of trans-anethole, the more delicate and sweet the odor and flavor of the oil (Guenther, 1950).

B. Caraway Seed Oil (Carum carvi L.)

There are two grades of caraway oil on the market: crude or natural oil which is the direct distillate, and double rectified or redistilled caraway oil. The crude caraway oil has an initial note of a nauseating almost amine-like type odor which may be due to either the decomposition of proteins in the seed germ or the presence of some glycosides or alkaloids. This odor is not present in redistilled caraway oil. The main use of caraway oil is in flavoring of food products such as meat, sausages, tinned goods, bread, cheese, pickles, sauces and seasonings. In addition, it is responsible for the flavor of the German liqueur kummel. Caraway seed oil is also used in mouthwash, gargle preparations, toothpaste flavors and chewing gums. It is often used in pharmaceutical preparations to overcome a bad odor and taste and as an aromatic carminative medicine. It has been reported that for flavoring, 100 lb of the best grade of dry caraway seed can be replaced by 5 lb

of oil, ("spice equivalent").

Caraway fruits are the fruit of Carum carvi L., a biennial plant native to western Asia and a large portion of northern and central Europe. It is also grown in the western United States and North Africa. Agrological data for growing the plant are presented in Appendix A-2. Besides the volatile oil which is the main constituent, caraway fruit contains fixed oil (triglycerides), cellulose, pentosans, calcium oxalate, mineral elements and protein (about 20%). The volatile oil, which gives the characteristic aroma and taste of caraway, is a colorless liquid which becomes yellowish on aging (Anonymous, 1970b; Guenther, 1950). Guenther (1950) recorded that the essential oil of Dutch caraway seed varied from 3 to 6% with an average of 4%, and the North African and Near East caraway seed oil from 0.4 to 1.0%. Sunny, dry weather during seed maturation reduced the oil content, but the oil was high in carvone and low in terpene hydrocarbons. The cool, damp weather increased the yield of oil but reduced the amount of carvone and other oxygenated terpenes. This difference might be due either to photochemical forces which transformed terpene hydrocarbons into oxygenated compounds or to the possibility that during sunny, hot weather, the more volatile constituents, particularly the terpene hydrocarbons, evaporate resulting in an increase of oxygenated compounds.

Gildemeister and Hoffman (1931) reported that caraway seed oil contained 50.0 to 60.0% carvone. Fritzche Brothers Inc. Laboratories (1950) recorded that genuine caraway seed oils from Holland and Russia contained 51.0 to 59.0% carvone, and oil from Ohio, 59.7%. Sandermann (1938) found 7.0% carvone and about 75.0% limonene in caraway oil of flowering plants. The oil also contained azulene and cadinene, which

was not present in caraway seed oil but in the oil from the leaves and stalks. Schimmel & Co. (1896) proved by large scale distillation tests that the carvone content of caraway seed oil depends upon the state of plant maturity, being highest in oils distilled from fully-matured seed. In the study of the carvone content of the developing fruits of Anethum graveolens L. and Carum carvi L., Betts (1965) found that caraway fruits contained little or no carvone one week after pollination; by three weeks 10 mg or more of carvone/100 fruits; and subsequent weeks between 12-20 mg carvone/100 fruits in 1962 and 11-14 mg carvone/100 fruits in 1964. The maximum yield of carvone could be obtained from the essential oil of 4-week old fruit which also contained a higher proportion of limonene than that of older fruits. This essential oil was sweeter, but less intensely flavored. He concluded that low carvone content in caraway oil did not reflect carvone deficiency in the fruit. The chaff resultint as a by-product in threshing and winnowing of the seed gives an oil with a harsh, inferior odor and flavor (Guenther, 1950).

Caraway oil contains 53 to 63% (w/w) of D*-carvone, together with D-limonene and small amounts of dihydrocarvone, carveol, dihydrocarveol, acetaldehyde, furfural, diacetyl, D-dihydrocarveol, L*-dihydrocarveol, L-neodihydrocarveol, L-isodihydrocarveol, L-isodihydrocarveol, D-perillyl alcohol and D-dihydropinel (Guenther, 1950). El-Deed et al. (1962a) used gas-liquid chromatography to analyze some essential oils. The chromatogram of caraway oil showed two peaks, limonene (45.58%) and carvone (56.898%). El-Deed et al. (1962b) used TLC and column chromatography to find that caraway oil contained carvone, limonene, α-phellandrene, α-terpinene and citral. By using TLC with 15% ethyl acetate in n-hexane

*d(+) or l(-) (or D,L) designations represent the optical rotations and do not essentially relate to the absolute configuration relative to D(+) glyceraldehyde

as a solvent they obtained two spots for caraway oil corresponding to carvone and limonene. In determining the monoterpene hydrocarbon composition of 29 non-citrus essential oils Ikeda et al. (1962) found that the total monoterpene hydrocarbon content of caraway oil was 38.0% of which d-limonene constituted near 100%, with traces of α-pinene, α-thujone, β-pinene, sabinene, Δ3-carene and α-phellandrene. By using gas-liquid chromatography to study Indian caraway, Atal and Sood (1966) found that the oil contained carvone, limonene, β-pinene and p-cymene.

Yin et al. (1970) studied the effect of acid and temperature treatments of caraway oil on the changes of its individual constituents. The components identified were α-pinene, β-pinene, myrcene, limonene, terpinolene, p-cymene, trans-dihydrocarvone, cis-dihydrocarvone, 1-p-menthen-4-ol, cis-8-p-menthen-1-ol, 8-p-menthen-2-ol (neo), 8-p-menthen-2-ol (iso), carvone, 8-p-menthen-2-ol (neo-iso), trans-carveol and cis-carveol. Two principal constituents, carvone and limonene, underwent minor changes when subjected to harsh conditions of pH and temperature, and the trace components changed when the oil was subjected to high temperature or to buffer-solutions of varying pH.

A good caraway oil contains 50 to 60% d-carvone, it being the most important constituent and the principal carrier of the characteristic odor (Anonymous, 1970b; Guenther, 1950).

C. Dill Seed Oil (Anethum graveolens L.)

Dill seed, either whole or ground, is used frequently as a condiment. It is utilized as a flavoring in pickling cucumbers and in Scandinavia for flavoring in bread, potatoes and vegetables. In France

the seeds are used extensively to flavor pastries and sauces, while in India it is used as a carminative and as an ingredient of curry. Chopped dill leaves, fresh or dried, are used on soups, salads, and such seafood as lobster or crayfish. The oil of dill herb is used for flavoring and seasoning purposes in the food industry and in the last ten years has largely replaced the whole herb. When compared with the best grade of dry spice herb, it is found that 1/2 lb of oil can replace 100 lb of herb.

Anethum graveolens L. grows wild in most parts of Europe, the Middle East, the Mediterranean coast of Africa, Indja and the Far East. The types of volatile oils obtained from Anethum graveolens L. are: (1) oil of dill herb or weed which is distilled from the herb along with the immature fruits; (2) oil of dill seed obtained from mature separated fruit. The two oils vary in composition and hence, in odor and flavor. Dill is grown in England and Hungary for the seed oil, in the U.S.A. and Hungary for the entire herb oil and in Germany and Holland for the weed oil (Virmani and Datta, 1970a; Guenther, 1950).

Dill is susceptible to weather hazards. Hail, strong winds and driving rain may injure the flowering plants resulting in no yield of oil (Guenther, 1950). The agrological requirements for growing the plant are summarized in Appendix A-3.

The oil content of dill seed varies from 2.1% in India to 5.62% in Russia and of dill herb from 0.16% of fresh weight in India to 1.5%of dry weight in Russia (Guenther, 1950; Virmani and Datta, 1970a).

The seed oil has a high d-carvone content which varies according to the plant geographical origin, species, size and ripeness of fruit.

The variation of carvone content due to geographical origins is shown by Guenther (1950), and Virmani and Datta (1970a). The percent (w/w) of carvone in dill seed oil was: Jammu and Kashmir 50.5 to 53.0, Jammu 45.9 to 52.0, Varikhaz 50.6, Katra 62.2, and Haldwani 41.83 to 56.6 (the foregoing were from India), Bulgarian oil 39, Irish 37.8, Hungarian 40.0 to 60.0, English 48 to 57, American 50.0 to 60.0, and Russian 53.6.

The varietal variation is shown by the composition of Indian dill varieties. The variation between the two varieties is not only in the carvone content but also in the other components. The seed oil of Anethum graveolens L. contains d-limonene (10%), β-phellandrene (6%), α-terpinene (6%), dihydrocarvone (12%), carvone (34.5%), carveol (4%), dihydrocarveol (3.5%) isoeugenol (2.3%) and dillapiole (3%) (Baslas et al. 1971). The composition of dill seed oil, Anethum graveolens L. sub. sp. sowa, was: α-pinene (5.0%), d-limonene (21.4%), d-phellandrene (11.4%), α-terpinene (3.6%), dihydrocarvone (14.3%), carvone (20.7%), caryophyllene (3.6%), myristicin (1.0%), eugenol (3%), apiole (5.7%) and dillapiole (8.5%) (Baslas and Gupta, 1971). Shah et al. (1971) analyzed the constituents of two varieties of Indian dill, Anethum sowa, and found that the variety Variyali sowa contained 21% carvone, 43% dihydrocarvone, 66% total carbonyl compounds, 13% dillapiole, and 20% limonene, while the second variety, Ghoda sowa, contained 35% carvone, 15% dihydrocarvone, 54% total carbonyl compounds, 12% dillapiole and 34% limonene.

The composition of dill seed oil varies with the size of the fruit. Grade A, which includes seeds with an average size of 4.5 mm in

length and 7.25 mm in width, has a 2.51% ester content and a 56.08% ketone content (calculated as carvone); for seeds of Grade B (3.60 - 4.50 mm length, 2.30 - 2.50 mm in width), 12.13% esters and 41.27% ketones; for the seeds of Grade C, which include seeds with an average size of 2.80 - 3.60 mm in length, 1.80 - 2.30 mm in width, 26.52% esters and 42.63% ketones; for seeds of Grade D (2.80 mm length, 1.80 mm width), 26.85% esters and 41.14% ketones (Gulati et al. 1969).

The d-carvone content increases with ripeness of the fruit. The very young fruit contains 17% carvone, 12% tertiary alcohols (expressed as linalool), 2% primary and secondary alcohols, 8% esters and 61% non-reacting compounds (probably monoterpene hydrocarbons such as limonene), while the ripe fruit contains 58% carvone, 7% primary and secondary alcohols, 18% esters and 17% non-reacting compounds (Luyendijk, 1957). In the study of the development of carvone in the fruits of dill and caraway Betts (1965) found that dill fruits initially contained 1 to 2 mg of carvone/100 fruits. Three weeks after pollination the carvone content was 5 to 9 mg/100 fruits in 1962 and 4 to 6 mg/100 fruits in 1964. The maximum carvone content was obtained from 4-week old fruit which also contained a higher portion of limonene than the more developed fruit. He concluded that the low carvone content of dill seed oil did not indicate the carvone deficiency in the seed.

The composition of dill herb oil also varies according to the geographical origin. This variation of carvone content according to geographical location is demonstrated by the results given by Guenther (1950) and Virmani and Datta (1970a). The percent (w/w) of carvone for dill herb oil is: India 22.0 to 39.39, America 12.0 to 40.0, Hungary 25.5 to 42.5 and Russia 15.

The composition of dill herb oil also varies according to crop year. In the analyses of the quality of dill seed and herb oil from Haldwani Gulati et al. (1969) recorded that the percentage content of dill herb oil was: for the crop year 1964, ester value 17.74 and ketone content 30.49 (calculated as carvone); for the crop year 1965, ester value 20.09 for leaves and umbel and 19.40 for whole plant, and ketone content 19.93 for leaves and umbels and 22.52 for the whole plant; for the crop year 1966, ester value 23.03 and ketone content 31.0.

The proper time of harvesting is of great importance because the quality of the oil depends mainly upon the state of maturity of herb and seed. The quality of dill herb oil depends on the amount of the two main constituents, phellandrene and d-carvone, the higher the amount of phellandrene, the more pronounced the odor and flavor of the fresh herb. The variability of carvone content in herb oil was due to the amount and stage of ripeness of the seed in the whole herb (Guenther, 1950). The CDA Research Branch at Morden, Manitoba (1972) reported the composition of dill herb oil at various stages of harvesting of their 1971 crop. The dill herb oil harvested on August 20 contained 1.1% α-pinene, 35.7% α-phellandrene, 28.0% limonene, 6.0% linalool and 26.4% carvone; that of August 25, 0.8% α-pinene, 28.4% α-phellandrene, 31.8% limonene, 4.8% linalool and 32.3% carvone; and that of September 21, 0.8% α-pinene, 25.8% α-phellandrene, 28.4% limonene, 4.8% linalool and 38.2% carvone. The dill herb oil obtained from Plum Coulee, Manitoba harvested on September 3 contained 1.2% α-pinene, 35.7% α-phellandrene, 37.1% limonene, 4.7% linalool and 18.0% carvone while the composition of the oil harvested on September 7 was 1.3% α-pinene, 37.6% α-phellandrene, 33.2% limonene, 4.7% linalool and 19.9% carvone. Guenther (1950)

summarized the effect of the state of ripeness of the herb material on the composition of dill herb. An oil distilled from partly seeding and partly flowering herb material contained 13.0 to 15.6% carvone. An oil distilled from herb harvested just after the flowering period contained 21.8% carvone. An oil distilled from green herb material harvested at the proper time of maturity and partly dried before distillation, contained 34.6% carvone. An oil distilled from half-matured and half-dried herb contained 45.8% carvone. An oil distilled from half-matured and completely dried herb had 52.0 to 58.0% of carvone. An oil distilled from dill seed and chaff contained 64.4% carvone. He recommended that the crop grown for the production of herb oil should be harvested when the most matured seeds are turning brown. The herb character still exists when the carvone content of the oil is less than 35.0% and is most pronounced when the oil contains 20.0% or less of this ketone.

Drying can also affect the composition of dill herb oil. Prolonged drying of the herb in the field would cause a considerable loss of oil from the evaporation of the more volatile terpene constituents. Thus, the oil distilled from dried herb would have a high carvone content, which is not desirable in herb oils. Such an oil resembles seed oil in respect to odor, flavor and composition (Guenther, 1950).

In their TLC study of the content and composition of some umbelliferous essential oils at different stages of growth, El-Hamidi and Ahmed (1966) found that dill oil produced six spots at the earlier and five spots at later stages of growth. The results showed that carvone and phellandrene were the major constituents. Reitsema (1954) used TLC with a solvent system of 10 to 15% ethyl acetate in hexane and

obtained for dill oil seven spots with Rf values of 0.03, 0.06, 0.11, 0.18, 0.33, 0.44 and 0.67.

D. Fennel Seed Oil (feoniculum vulgare Mill.)

Fennel seeds are aromatic, with a warm, sweet odor somewhat similar to that of anise, and have a slightly burning flavor. Fennel oil is used as a flavoring agent in culinary preparations, bread, pastry, confections, meat, pickles and non-alcoholic beverages. The spice equivalent of this oil is 5 lb, i.e., this amount is able to replace 100 lb of the best grade dried fennel seed.

Of about a dozen varieties of fennel plant, Feoniculum vulgare Mill. is the most important one. Feoniculum vulgare Mill. is a herb native to Europe and the Far East. There are two main subspecies of the plant, the bitter fennel Feoniculum vulgare Mill. var. vulgare and the sweet or Roman fennel, Feoniculum vulgare Mill. var. dulce. Bitter fennel is grown throughout Europe, Asia and America. Sweet fennel is cultivated around the European Mediterranean coast (Guenther, 1950). The agrological requirements for growing this plant are summarized in Appendix A-4.

Fennel, a perennial plant, usually produces only a few seeds during the first year of growth, but a full crop in succeeding years. The seed should be harvested before it is fully ripe when it is sufficiently hard, and greenish-grey in color. The oil yield of the best grade seed from eastern Europe is between 4.0 and 5.0%, and the lowest yield is 2.5%. The older seeds give a lower yield (Guenther, 1950). The oil yield is found to be different between the two varieties. The sweet fennel seeds yield 1.50 to 3.82% volatile oil, while bitter fennel seeds yield 2.92 to 5.76% oil (Karlsen et al. 1969). The sweet fennel

seeds from Nigeria yield 2.0 to 2.4% oil (Osisiogu, 1967). The seed of Ooty fennel, Feoniculum vulgare Mill. sub. sp. vulgare, was reported to yield 8.5% volatile oil (Shah et al. 1970).

The composition of fennel oil varies according to the geographical origin. By using the method of congealing points Guenther (1950) found that the amount of anethole from fennel oil from Minnesota was 65.0%, and from Russia, 61.0 to 62.5%. The anethole content of sweet fennel oil from southern France ranged from 83.0 to 87.0%. The anethole content of fennel oil from Nigeria analyzed by gas-liquid chromatography was 86% (Osisiogu, 1967). The CDA Research Branch in Morden, Manitoba (1972) reported the following composition of locally grown fennel: 3.4% α-pinene, 1.4% α-phellandrene, 47.1% limonene, 3.2% linalool, 2.7% anisol, 0.9% carvone and 36.2% geraniol.

The composition of fennel seed oil also varies according to the plant varieties. This varietal difference is shown by the content of anethole, the only major and the most important constituent of fennel oil. Sweet fennel oil contains 75.0 to 87.0% of anethole while bitter fennel oil has 61.0 to 75.0% of the compound (Guenther, 1950). Fenchone was reported to be present in bitter fennel seed oil but not in sweet seed oil (Guenther, 1950). Recently, it was found that both sweet and bitter fennel seed oils contain fenchone (Betts, 1968; Karlsen et al., 1969). However, sweet fennel seed oil has a lower amount of fenchone than bitter seed oil. In sweet fennel oil the fenchone content is in the range of 1.3 to 11.1 parts and in bitter fennel seed oil from 16.3 to 31.6 parts relative to 100 parts of trans-anethole (Karlsen et al., 1969). The varietal difference is also observed in the other constituents. Shah et al. (1970) reported that Ooty fennel seed oil contained

estragole as the major constituent and no anethole. The estragole content in sweet fennel seed oil varies from 3.0 to 5.0, and in bitter seed oil from 2.6 to 5.5 relative to 100 parts of trans-anethole (Karlsen et al., 1969). Both varieties contain seventeen monoterpene hydrocarbons. The percentage composition of monoterpene hydrocarbons in sweet and bitter fennel is as follows: α-pinene 0.60 and 14.90, α-thujone 0.05 and 0.06, fenchone 0.00 and 0.06, camphene 0.05 and 0.09, β-pinene 0.12 and 0.65, Δ3-carene 0.45 and 30, α-phellandrene 0.16 and 1.25, myrcene 0.60 and 3.60, limonene 1.40 and 8.60, α-terpinene 0.20 and 0.30, β-phellandrene 0.55 and 1.75, an unknown 0.25 and 0.50, γ-terpinene 2.20 and 1.20, cis-ocimene 2.50 and 0.00, terpinelene 0.70 and 0.30, trans-ocimene 0.02 and 0.15, and p-cymene 1.00 and 0.50, respectively.

The composition of fennel seed oil varies with the maturity of the seed. Betts (1968) studied the anethole and fenchone contents in the developing fruits of bitter and sweet fennel varieties over three growing seasons. He observed that in both varieties anethole content continued to accumulate to about 22 mg/100 seeds. Fenchone was found at all stages of development in both varieties and was increasing continuously to about 10mg/100 seed in the bitter variety and to about 2 mg/100 seed in the sweet variety. In the study of the composition of brownish-green and green fruits of sweet fennel Karlsen et al. (1969) found that estragole content relative to that of anethole was almost constant, and that the fenchone content was much higher in green fruits. The fenchone content in brownish-green fruits ranged from 2 to 5 parts, and in green seed from 11 to 14 parts relative to 100 parts of trans-anethole. The difference in composition as shown by TLC of bitter fennel seed oil distilled at different stages of development was

reported by El-Hamidi and Ahmed (1966). The chromatograms showed five spots for the oil obtained at the earlier and ten spots at the later stage of fruit development.

The quality of fennel seed oil depends on two constituents, anethole and fenchone; the higher the amount of anethole and the lower the amount of fenchone, the better the quality of the oil. Bitter fennel seed oil of good quality should contain 50.0 to 60.0% of anethole. Fenchone, which is present in higher amounts in bitter fennel oil, causes a somewhat coarse and bitter taste. Due to the high amount of anethole and low amount or absence of fenchone, sweet fennel seed oil has a more delicate and sweet odor and flavor (Guenther, 1950).

E. Peppermint Oil (Mentha piperita L.)

1. Uses and Oil Yield

Peppermint is certainly one of the most popular flavors. Oil of peppermint is used in the food industry for flavoring a wide range of consumer products such as:

non-alcoholic beverages	99 ppm
alcoholic beverages	240 ppm
ice cream, ices, etc.	110 ppm
candy	1,200 ppm
baked goods	300 ppm
gelatins and puddings	75-200 ppm
chewing gum	8,300 ppm
meats	8 ppm
icings	5-54 ppm
toppings	650 ppm

Peppermint oil is also used in cosmetics, toothpaste, mouthwash, tobacco flavoring and for masking objectionable taste and smell of medicinals.

Mentha piperita L. is a native of Mediterranean countries. Peppermint grows wild in damp places in Europe and North America from Nova Scotia to Minnesota, and south to Florida and Tennessee. It is extensively cultivated in southern Michigan, northern Indiana, Oregon, Washington and Ohio. The two commercially important varieties are the black or English mint, M. piperita L. var. vulgaris, which is extensively grown in the Mid-West of U.S.A., and American mint, M. piperita L. var. americana, which is as hardy as the first variety but gives a lower yield of oil. The less commercially important white mint, M. piperita L. var. officinalis, grown around Mitcham, Surrey, England is a less hardy plant. However, the oil distilled from this variety is considered the finest of all commercial peppermint oils (Guenther, 1952).

The oil yield depends a great deal upon the condition of the fields, plant age, absence of diseases, pests, weeds, climate, etc. In America the average yield ranges between 0.3 to 0.4% on the dry basis, but may be as high as 1%. The highest yield, 5.3% on the dry basis, was recorded in Russia (Guenther, 1952; Virmani and Datta, 1970b). Ellis et al. (1941) observed that in most cases the yield of oil increases as the herb approaches maturity, i.e., full bloom, after which the yield decreases rapidly as the foliage begins to fall. Sardanovsky (1929) reported that the oil content of the leaves is highest during the interval between inflorescence and opening of the blossoms. By studing the relationship of oil yield and free menthol content, Ellis and Gaylord

(1944) observed that the oil content in the plant increased up to a certain point at which the free menthol content reached a value of 45%. However, when the plant was not harvested at this point, the yield of oil decreased, though the free menthol content still increased.

It was observed that the quality and yield of the oil are influenced by: 1) exceptionally high luminosity, 2) the long growing season, 3) the length of the day during the height of the season, from 16 to 18 h. Under these conditions the oil yields for Yakima Valley, Washington were in a range of 73 to 168 kg/ha* and the average annual yield was 84 kg/ha (Virmani and Datta, 1970b). The agrological requirements for growing this herb are listed in Appendix A-5.

Since it is difficult to achieve complete removal of the oil from fresh mint herb by steam distillation, the more facile distillation of an oil of slightly inferior quality from cured herbs is widely practiced. However, drying for a long period of time in direct sunlight causes a substantial loss of oil resulting from factors such as shattering of leaves, resinification and possible evaporation of the oil (Guenther, 1952). During the distillation, 40 to 45% of the oil is obtained within the first 10 min after which the amount of oil being distilled decreases as the distillation proceeds (Ellis et al., 1941).

2. The Effect of Some Agrological Treatments Upon the Yield and Quality of Peppermint Oil

As early as 1926, Maku reported that nitrogen (N) improves the growth and increases the oil yield. However, in comparison to N, potassium (K) and phosphorus (P) were more effective in increasing the oil yield. According to Springer (1937), the oil yield depends on the quality of the

*ha = 2.471 acres

soil and fertilization. He observed that NP fertilizers gave the best result. However, the fertilizer treatments did not influence the overall chemical composition of the oil. Schlemmer and Springer (1939) found that the highest yield of herb is obtained when either a mixture of superphosphate and potash or superphosphate and ammonium sulphate is used; however, there is no appreciable increase in oil content. According to Ellis et al. (1941), fertilizer applications affected the maturity of the plants but not the chemical properties of the oil. Birkeli (1948) observed that the black mint required larger quantities of K and N and lesser amounts of P.

In laboratory tests it was found that increasing N from 0.15 to 1.20 g for each plant grown in quartz sand, increased the oil content from 1.4 to 2.6%. However, the increase of P resulted in a smaller increase in oil content and absolute yield, while the addition of K resulted in a lower content of oil (Schratz and Wilmann, 1949). In field plot experiments Khotin (1950) observed that fertilization of soil with sodium nitrate and ammonium sulphate nearly doubled the oil yield. The effect is more pronounced when fertilization is done in the early stages of plant growth.

The N fertilization results in a marked increase in herb and oil yields, while P fertilization results in a small increase in the yields. Both K and sulfur (S) produce negligible effect on the yields (Baird, 1957). According to Steigerwald (1959), magnesium (Mg) increases the herb yield, though the oil content is not significantly affected. Latypov (1960) observed that fertilizer treatments increase the plant oil content from 60 to 85% and improve the oil quality. NH_4-fertilizer is more effective than nitrate fertilizer, while K_2SO_4 and $(NH_4)_2SO_4$ are more effective than NH_4Cl in increasing the oil yield.

In an extensive study on the effect of commercial fertilizers on the oil yield of mint, Green (1963) found that fertilizers in general bring about an increase in yield. The type of fertilizer and the rate of application depended on the type of soil, preceding crop and management practices. Thus, on highly organic muck soils, 335 to 560 kg/ha of N5, P20, K20 fertilizer was applied to first-year mint. In cool, wet seasons 30 to 55 kg of N/ha was applied as a side dress to give young plants a better start. On established plantings 280 to 560 kg/ha of N5, P20, K20 fertilizer or equivalent was applied before growth. On sandy loam soils, the equivalent of 135 kg of N/ha was applied, usually as ammonium sulphate or nitrate. The use of K and P was justified only when soil analysis indicated a deficiency in one or both nutrients.

In the study of the effect of fertilizers on the yield of herb and composition and yield of oil, Skrubis (1964) observed that N at the level of 14.3 g/m^2 had a significant effect on the yield of fresh herb of the first harvest, while P at the level of 20 g/m^2 and K 17.3 g/m^2 as well as their combination, had no significant effect on the yield of fresh herb. In the first harvest the yield of essential oil was increased by N and K and in the second harvest by N, P, K and NP treatments. However, the composition of the oil was not affected by these fertilizers. O'Connor (1965) studied the effect of N, P and K alone or in combination on the yield and the quality of the oil and found that high amounts of N increased the yield of oil significantly but reduced its menthol content. Similarly, superphosphate decreased the menthol content, while NPK reduced the oil yield.

Under greenhouse conditions a treatment of the seedline with plant hormones such as amido-α-naphthyl-acetic acid increased the

menthol and oil contents in the leaves. When the seedling was soaked in the solution (10^{-4} to 10^{-5}%) and then transplanted, the oil content in the leaves increased from 29 to 46%, and the menthol content from 4.5 to 9.0%. In such treatments the higher concentrations of hormone were more effective (Maciejewska-Potapczykowa and Kaminska, 1956). By sprinkling the plant with Na-α-naphthyl acetic acid or its methyl amide in concentrations of 10^{-4} and 10^{-5}% similar increases in the amounts of oil and menthol were obtained. The oil yield increased by 28 to 54%, and the menthol content by 4.9% (Moycho et al., 1954). When the plant was sprayed with 0.001 or 0.01% solution of gibberellin, it grew significantly taller (Kirsyte, 1965). There was a substantial increase of leaf, stem and flower weights but the oil content in the leaves dropped by 7-10%. Gjerstad (1960) treated the plants five times over a period of three months with a foliar spray of aqueous gibberellic acid and found that the oil content decreased by 52.4%, however the menthol content was the same as in the control.

3. Composition of Peppermint Oil

The composition of oils distilled in Ontario and United States--Mid-West, Oregon and Yakima as well as Europe, South Africa and Argentina was studied in detail by gas chromatographic analysis. Besides the monoterpene hydrocarbon constituents, most of the menthone-related constituents including the stereoisomers were reported. The results of these analyses and their comparisons are tabulated and presented in Appendix D-1.

Regardless of the geographical origin, the major constituents of the oil are l-menthol and l-menthone. The stereoisomers of these constituents, neomenthol and isomenthone, are always present. Also, the

acetic acid ester of menthol is always present. The other menthone related constituents present in the oil are menthofuran, pulegone and piperitone. The only major monoterpene hydrocarbon constituent present is limonene. The monoterpene hydrocarbons always present in smaller amounts are α- and β-pinene, while the presence of camphene is dependent on the oil origin. Many of these constituents might be used to assess the geographical origin or the plant species used in oil production. Thus, the ratios for menthone/isomenthone, limonene/cineol, neomenthol/menthyl acetate, menthol/neomenthol, etc. were found to be useful for identifying the origin of the oil. However, these ratios are typical for natural oils, and are different from similar ratios of redistilled, rectified, dementholised, deterpenised oils also found on the market either as improved or as adulterated oils (Smith and Levi, 1961).

Recently, Lawrence et al. (1972) reported that oil from Oregon contained a total of 99 constituents most of which were present only in traces. A detailed study of the monoterpene constituents present in peppermint oil was also reported recently by Hefendehl and Murray (1973). A total of twenty-five constituents were identified, with limonene the only major constituent.

The influence of stage of harvesting on the composition of oil was first observed on American peppermint oils by Kleber (1914). He reported that the plant first develops l-menthone which in later stages seems to be reduced into menthol. In a study of Russian peppermint oil Rutovski and Travin (1929) confirmed that the menthol content increases while menthone content decreases with the plant maturity as shown in the summary below.

Date	Stage of Development	Menthol Ester%	Menthol Free%	Menthol Total%	Menthone %
July 17-19	Long before blooming	8.21	39.67	47.88	13.04
Aug 8	Without buds	7.25	44.85	52.10	
Aug 14	With buds	7.97	46.07	54.04	6.38
Aug 31-Sept 3	Beginning of blooming	11.16	48.82	59.98	7.22
Sept 17-18	Full Bloom	13.04	46.33	59.73	1.43
Sept 26-Oct 3	End of blooming	15.68	45.00	60.68	2.46

The observation made by Sardanovskii (1929) also agrees with the above results. He reported that the percentage of menthol in the oil increases with the growth of the plant and attains a maximum near the end of blooming. In a study of the relationship of oil yield and free menthol content in the oil, Ellis and Gaylord (1944) found that the oil yield of the peppermint plant increases up to about 45% free menthol, and, when the plant is allowed to stand, the yield of oil decreases and the free menthol content continues to increase. This increase seldom exceeds 60%, though the total menthol, which includes the free and esterified menthol, exceeds this percentage. The oil which contains an appreciable portion of blossom oil was found by Watson and John (1955) to usually be rich in menthofuran. Since this terpene has a hay-like odor and unpleasant flavor, they associated the poor quality of the oil with the presence of a high amount of menthofuran. In a study of the occurrence of menthofuran in oil, Lemli (1957) found that menthofuran is secreted in the young leaves and buds of the plant, where metabolism is most active. The oil distilled from seven-day old plant possesses the highest content of menthofuran. The content decreases as the plant develops, but increases again from the time of flower bud formation and finally decreases

after flowering. A three-year study on the major constituents of peppermint oil in relation to the maturity of the plant revealed that the esterified and free menthol contents continue to increase with maturity of the plant, while the menthone content decreases even after the blossoms have gone (Watson and John, 1955).

It was observed that the length of curing negligibly affects the oil composition (Watson and John, 1955). The amounts of free and esterified menthol increase as distillation proceeds, while the menthone content decreases. Therefore, in order to obtain a good quality oil with high content of free and esterified menthol the herb should be distilled completely (Ellis et al., 1941).

The characteristic of peppermint oil flavor is apparently due to the combined effect of several constituents in the oil (Pintaure, 1971). A good quality oil should be high in menthol and menthyl acetate (Guenther, 1952; Virmani and Datta, 1970b). However, of the four stereoisomers, menthol, neomenthol, isomenthol and neoisomenthol, only menthol gives an agreeable odor of peppermint. The presence of a high amount of neomenthol, isomenthol and neoisomenthol imparts a "musty" or "flat" odor to the oil (Hornstein and Teranishi, 1967; Arctander, 1969). Menthone is also a characteristic and valuable component of peppermint oil (Pintauro, 1971), but due to its bitter taste, excessive menthone content is undesirable (Guenther, 1952). The association of poor peppermint oil quality with high menthofuran content was first reported by Watson and John (1955). The effect of menthofuran content on the flavor of peppermint oil can be detected significantly at 9.5% and higher (Cash et al., 1971).

F. Sage Oil (Salvia officinalis L.)

In the food industry the bulk of sage oil is used for the flavoring of baked goods, table sauces, canned and packed foods, soups, meats (especially sausages), pickles, candies, chewing gum and finally, for flavoring non-alcoholic beverages. The amount of sage oil which can replace 100 lb of the best grade of dried herb is reported to be 2 lb.

The true Dalmation sage is a native of the Dalmation Islands and the adjacent coast of the Adriatic sea. Of a wide range of varieties of Salvia officinalis L., the Dalmation sage possesses the finest and the most characteristic aroma. American sage grows well in a region from central Georgia, south to Wisconsin and throughout the eastern coastal states. It is now being grown mainly in the state of Washington (Guenther, 1952). The agrological requirements for growing this herb are listed in Appendix A-6.

Young plants that have not reached the flowering or seeding stage contain a high amount of an oil with a fine aroma. The average herb oil yield of Dalmation sage decreases from 2.0% at the beginning of the harvest to about 0.7% at the end of the harvest, and the average yield for the growing season is 1.4%. In the case of American sage the oil yield is reported to range between 0.6 and 1.0% (Guenther, 1952).

The composition of genuine Dalmation sage oil was reported by Guenther (1952). The so-called "High Test Oil", with high thujone content, contained 1.6 to 4.0% esters calculated as bornyl acetate, 6.9 to 16.0% alcohols calculated as borneol and 41.6 to 61.2% ketones. The "Low Test Oil" contained 2.2 to 4.9% esters, 11.6 to 15.0% alcohols and

22.0 to 39.7% ketones. Recently, Brieskorn and Wenger (1960) reported the composition of Dalmation sage oil as follows: 3.3% α-pinene, 5.6% β-pinene, 14.8% cineol (linalyl acetate), 51.0% thujone, 8.2% camphor, 6.6% borneol, and 1.7% bornyl acetate. The composition of Dalmation sage varies according to stage of harvesting and the growing season (Guenther, 1952). The oil distilled from whole flowering plants grown in summer contained 32.0 to 35.0% cineol, about 30.0% sesquiterpenes, 9.0 to 14.0% borneol, 5.0 to 10.0% thujone and camphor and 2.0% esters. The composition of sage oil distilled from the whole non-flowering plant grown in winter was 13.0 to 20.0% cineol, about 20.0% sesquiterpenes, 7.5 to 12.0% borneol, 20.0 to 32.0% thujone and camphor, about 15.0% monoterpene hydrocarbons and 2.2 to 3.7% esters.

The composition of sage oil obtained from other regions is also reported. Italian sage oil contained 15.0% terpenes, 31.5% ketones (α-thujone and L-camphor), 15.0% cineol, 11.2% free alcohols (L-borneol and probably DL-borneol), 2.3% esters and 20.0% sesquiterpenes. Three good quality sage oils from the state of Washington contained 3.3 to 6.0% esters calculated as bornyl acetate, 13.0% alcohols calculated as borneol and 35.4 to 46.7% ketones calculated as thujone. In the analyses of essential oils of some labiaceous plants from Egypt, Karawya et al. (1970) reported that sage oil contained 2.472% α-pinene, 3.708% sabinene, 7.879% β-pinene, 11.433% camphene, 2.781% myrcene, 9.88% limonene, 18.54% β-phellandrene, 13.348% ocimene, 0.309% p-cymene, 17.119% cineol, 2.161% borneol, 0.742% camphor, 0.730% linalool, 0.249% bornyl acetate and a trace of terpineol. The TLC of this sage oil using ethyl acetate: petroleum ether (40°-60°) 15:85 as the solvent revealed six spots. The Rf values and identities of these spots were as follows: 0.35-terpineol,

0.50-camphor, 0.47-linalool, 0.71-1,8-cineol, 0.74-geranyl acetate and 0.96-terpene hydrocarbons. In the characterization of essential oils by TLC using 10 to 15% ethyl acetate in hexane as solvents Reitsema (1954) found that sage oil produced five spots with Rf values of 0.22, 0.31, 0.54, 0.63 and 0.94. Finally, the CDA Research Branch, Morden, Manitoba (1972) reported that the oil from locally grown sage contained 5.5% α-pinene, 2.6% α-phellandrene and 10.4% anisol, while that of broad leaf sage, also locally grown, contained 4.5% α-pinene, 1.8% α-phellandrene, 14.9% anisol, 0.7% carvone and a trace of geraniol.

Thujone is the most important constituent of sage oil and is largely responsible for its characteristic odor and flavor. A higher percentage of thujone indicates a good quality (Guenther, 1952).

G. Thin-Layer Chromatography

In the analysis of essential oils thin-layer chromatography (TLC) is widely used to screen the minor and major constituents of these oils, and to identify these constituents with or without coupling to a GLC. Scott (1973) gave advantages of adsorption TLC over column chromatography where the separation of a large amount of material is not important. The relatively easy spreading of adsorbent on glass plates replaces the tedious packing of columns. TLC is important when the compounds under study are available in small amounts such as in the case of essential oils. It is cheap, rapid and gives good resolution. The separated constituents can be easily detected directly by visible or UV light, or can be readily visualised by detection reagents. Furthermore, measurement of Rf values is more convenient than measurement of retention volumes as in column chromatography. When compared with the latter separation,

the TLC technique has a number of advantages the most important of which are versatility and sensitivity (Kirchner, 1973).

Based on a survey of 1,107 literature reports, Scott (1973) tabulated the frequency of the adsorbents used in TLC as follows: silica gel G 53.8%, silica gel H 10.1%, alumina 3.1%, cellulose 8.9%, kieselguhr 0.6%, polyamide 2.4% and others 21.1%. In addition, he stated that silica gel (silica, silicic acid and kieselgel), unlike alumina, does not catalyze chemical alteration of labile samples. He also found that the range of sample concentration over which linear isotherm separation (where Rf values are constant) can be performed for silica gel is 10-fold greater than for optimally prepared alumina and 100-fold when compared to the highly activated chromatography grade alumina.

Silica gel has a polar and slightly acidic surface; hence, it preferentially adsorbs polar and basic compounds. It possesses a total surface area ranging from 300 to 600 m^2/g. On the surface of silica gel there are three significant chemical species: -Si-OH (free hydroxyl), -Si-O-Si- (siloxane) and -Si-OH...OH-Si- (reactive hydroxyl). Of these, the siloxane is considered the least important in adsorption. For compounds with multiple binding sites, the reactive hydroxyl is the strongest adsorbent site. However, for compounds with a single functional group, both free and reactive hydroxyls are equivalent. Reactive hydroxyls are located in higher concentrations at smaller pores thus contributing less to the properties of typical wide pore TLC silica (Scott, 1973).

Silica gel for TLC usually contains a binder, either starch or calcium sulphate (gypsum), to enhance adherence of the stationary phase

to the plate. Scott (1973) found that this binder does not exert extensive influence on the character of the adsorbent. The impregnation of silica gel with ferric oxide, mercuric acetate, thallous or silver nitrate has been reported. By using silver nitrate impregnation of silica gel Lawrence (1968a) was able to separate unsaturated terpenes according to the position and number of double bonds. The separation was achieved due to the ability of the unsaturated compounds to form π-complexes with silver ions. He concluded that cyclic terpenes with single internally located double bonds do not readily form complexes, that cyclic or acyclic terpenes with two non-terminal double bonds do not readily form complexes unless the double bonds are in cis conjugation, and that cyclic or acyclic terpenes with exocyclic or terminal double bonds readily form π-complexes with silver ions. Recently, using a thallous nitrate impregnated adsorbent, Baines and Jones (1970) established that the stability of the complex is increased in the following order: bicyclic terpenes with a single internal double bond, bicyclic terpenes with an exocyclic double bond, monocyclic terpenes with two non-terminal double bonds, and monocyclic or acyclic terpenes with exocyclic or terminal double bonds. However, due to its toxicity, thallous nitrate was not accepted widely as an impregnating agent. Impregnating with mercuric acetate brought about a similar separation as silver nitrate, although compounds with two internally located double bonds did not form stable complexes, even when the double bonds were cis conjugated (Lawrence, 1971). By using silver nitrate impregnated silica gel layers, Stahl and Vollmann (1965) separated terpene alcohols with an equal number of carbon atoms on the basis of double bonds and found that 2.5% of silver nitrate was the optimum content for the separations.

Similarly, Ikan and Meir (1965) were able to separate oxygenated terpenes such as geraniol, citronellol, carvone, pulegone, citral and piperonal on silica gel layers impregnated with silver ions. A comprehensive review of the adsorbents applied in the analysis of essential oils and their constituents has been given by Stahl and Jork (1969).

The thickness of adsorbent is also of importance in TLC analysis. Stahl (1969) has shown that plates with a very thin layer of adsorbent developed more slowly. The rate of development improves with increasing layer thickness until 250 μ beyond which no gain is observed in development rate. A thicker layer was necessary where greater capacity of the stationary phase was needed to isolate individual constituents for further identification tests as in preparative TLC. (Stahl, 1969; Bobbitt, 1968). The thickness of the layer within the range of 0.15 to 2.0 mm does not affect significantly the Rf values and the efficiency of separation. Thin layers are best suited for diagnostic or qualitative analysis, and the majority of such analyses have been carried out with layers of 0.25 mm thickness (Bobbitt, 1968). When it is stated that 250 μ layers are used, it means that the spreader gate is set at 250 μ. The real thickness of the adsorbent is actually thinner because of the shrinkage which occurres as water evaporates during activation. The degree of shrinkage varies with the initial water content in the adsorbent slurry, actual particle size, and the type of adsorbent used (Scott, 1973).

Before TLC separation the plates are activated, usually at 105°. Scott (1973) stated that in a silica gel slurry there is capillary water as well as water associated with hydroxyl groups. The water bound to hydroxyls must be removed during the activation because it tends to

resist the movement of less polar components during separation. Thus the activation of the plates actually exposes the reactive sites of the adsorbent, allowing them to interact with the compounds to be analyzed. He also established that optimal activation is achieved when the plate is heated at 105° to 110° for 30 min. Stahl (1969) claimed that a very active layer can be obtained by heating silica gel plates for 3 to 4 h at 150°. However, such a high activation appeared to be necessary only when the substance is applied in a dry atmosphere and when anhydrous solvents are used. On the other hand there is a danger that the substance might decompose on a highly active layer. He also indicated that without previous drying of the plates at room temperature, it takes nearly 2 h for the plate to be activated in a oven at 110°. Predrying at room temperature reduces the time of activation. As found by Scott (1973), heating at 150° to 200° removes the capillary water, while at 200° to 400° the loss of additional water is due to the conversion of hydroxyl groups to the siloxane structure. Heating above 200° converts the silica gel into an adsorbent with preferential adsorption characteristics for the presence of functional groups or unsaturation degree of the compounds analyzed.

The solvent system used in TLC separation is able to contribute further in the separation of terpene mixtures. A detailed list of the solvent systems used for various essential oils and terpenes was given by Stahl and Jork (1969). In addition, Lawrence (1968b) gave his list of the 'Top Ten' solvent systems in TLC of essential oils which included: benzene, benzene:ethyl acetate (95:5), hexane:ethyl acetate (85:15), hexane, benzene:ethyl acetate (90:10), chloroform:benzene (1:1), chloroform, hexane:ethyl acetate (85:15), benzene:ethyl acetate (85:15), and

hexane:ethyl acetate (90:10). The same author also confirmed the earlier findings that systems which are a mixture of high and low polarity solvents, such as hexane:ethyl acetate (85:15), give less diffuse spots in the TLC separation of essential oil constituents than low polarity solvents, either mixed or alone.

Numerous spraying reagents have been used in detecting the constituents of essential oils. Among those widely used are: sulphuric acid and ammonium hydrogen sulphate for detection of all organic constituents, antimony tri- and pentachlorides, phosphomolybdic acid, vanillin-sulphuric acid, and p-dimethylaminobenzaldehyde-sulphuric acid as general terpene reagents; diphenylpicryl hydrazyl, fluorescein-bromine, iodine and potassium permanganate for unsaturated terpenes; and finally, for essential oil phenol constituents, phloroglucinel-sulphuric acid, Folin-Ciocalteu reagent and ferric chloride (Lawrence, 1968b). Stahl and Jork (1969) claimed that terpenoid substances are best visualized with the anisaldehyde-sulphuric acid, or antimony chloride reagents or molybdophosphoric acid. They also classified terpenoid compounds into various groups, each with its own spraying reagent. For visualization of mono- and sesquiterpene hydrocarbons sulphuric acid or anisaldehyde-sulphuric acid reagent was used. Terpene oxides, and peroxides were visualized with anisaldehyde-sulphuric acid, antimony tri- and pentachlorides and acidic p-dimethylaminobenzaldehyde reagents. The detection of terpene esters and lactones was found by the above authors to be achieved by molybdo-phosphoric acid, anisaldehyde-sulphuric acid and antimony tri- and pentachloride reagents. Furthermore, terpene aldehydes and ketones were visualized most often with an acidic 2,4-

dinitrophenylhydrazine reagent, however, camphor detection appeared to be more sensitive using Dragendorff reagent. The detection of mono- and sesquiterpene alcohols was achieved with molybdophosphoric acid reagent which appeared to be a sensitive, though non-specific reagent. For the same alcohols other reagents such as anisaldehyde-sulphuric acid, antimony tri- and pentachlorides were also used. However, the detection with anisaldehyde-sulphuric acid reagent proved to be more sensitive than that with antimony chlorides.

Kaiser (1969) regarded the coupling of TLC-GLC as a new analytical entity which can supply superior information on the analysis. The GLC-TLC coupling allows a double and a two-dimensional separation. Janak (1963) observed that with this coupling procedure it is possible to separate the compounds according to the number of carbon atoms with GLC and the type of functional groups with TLC. Kaiser (1969) mentioned that this procedure allows a multiple identification as well as a quantitative determination of individual components. It is a most critical control procedure which reduces errors in qualitative results and checks the quantitative values. This coupling technique also yields further information on the sample constituents, the chemical changes which may occur during GLC or TLC analysis, and the errors in the GLC quantitative response. He also observed that discontinuous coupling of TLC-GLC is time consuming, and may be accompanied by the loss of components and the loss of information concerning the structure. Neill et al. (1964) stated that this technique is sufficiently accurate for biological analytical problems with as little as 10^{-7} g of substance.

H. Gas-Liquid Chromatography

1. Column

Most of the analyses of essential oils are carried out by using large diameter packed columns. Lawrence (1971) stated that these large columns do not give adequate separations. Teranishi (1970) recommended the use of capillary open tubular, or small diameter packed columns for more efficient separations. Sometimes a large diameter preparative column is used in conjunction with a capillary column.

Zubyk and Conner (1960) established that isomerizations are caused by copper columns but not by glass and steel columns. Similarly, copper can also catalyze the decomposition of many volatile oil constituents (Humphrey, 1970). The stainless steel and aluminum columns cause extensive adsorption of polyols and vanillins while copper and glass columns do not exhibit this effect. However, by coating the metal walls with a thin film of a polar stationary phase, the adsorption might be eliminated (Levins and Ottenstein, 1967). In addition Ottenstein (1973) found that metal columns or any metal in the chromatographic system can cause tailing of peaks. Ziegler and Guenther (1971) compared the use of glass columns with metal columns in the analysis of terpene hydrocarbons, alcohols, aldehydes, esters and sesquiterpenes present in citrus fruit essence and established that the glass column is superior, giving better separation, high recovery of the alcohols and esters, negligible formation of artifacts, and a possibility of temperature programming over 180° thus permitting the separation of high boiling sesquiterpenes and furocoumarines. Jennings (1972) stressed that hot metal, not only of the injector port but also from the column itself, can catalyze changes

that result in separations which are impressive but meaningless, because the compounds on the chromatogram bear little relationship, qualitative or quantitative, to compounds originally present in the product. Glass-lined injectors and glass columns can overcome these problems. They also minimize on column reactions and permit the observation of packing defects, discoloration of the liquid phase due to degradation, and void space that develops in the column during use, all of which can produce confusing or even erroneous results.

Huyten et al. (1960) found that the height equivalent to theoretical plate (HETP) of a large diameter column increases linearly with the length of the column until the influence of radial diffusion becomes important, resulting in a constant plate height independent of further column lengthening. This constant value is reached after about 1 m for loose packings and a few meters for dense packings. Short length columns connected by narrow tubing give some improvement. Giddings (1964) showed that the resolution of neighboring peaks increases with column length. However, the main limitation in column length increase is the pressure drop in the system. Karger and Cooke (1964a) also emphasized that there is an optimum length for a set of operating conditions. For instance, a shorter column produces better resolution in a shorter time at lower temperature than a long column. Since the shorter column can be operated at a lower temperature, decomposition may be avoided. The capacity factor determines the optimum length and further increase from this length causes a decrease in resolution. A small capacity factor results in shorter optimum column and a greater decrease in resolution. The rate of decrease in resolution is found to be more severe for capillary than packed columns because of a smaller capacity factor in the former.

Jennings (1972), however, stated that a glass capillary column 65 m in length with an inner diameter of 0.025 mm, shows an efficiency of 8 - 50 x 10^4 theoretical plates and is able to give a separate peak for almost every component of a mixture. Verzele et al. (1972) reviewed the advantages and disadvantages of a capillary column, and found that the combination of wider bore glass capillaries with a technique of on-column injection simplified the use of open tubular capillary columns.

2. Solid Support

Using scanning electron microscopy Drew and Bens (1968) studied the solid supports used in GLC and found that their surface characteristics are complex, usually consisting of porous granular particles with a high surface asperity. Conner (1958) reported that the isomerization of α-pinene to camphene occurred on pink (P) but not white (W) solid support. Pink support was found by Holmgren (1958) to cause dehydration of certain tertiary and α and β unsaturated primary alcohols. Scholz and Brandt (1962) found that Chromosorb P absorbs roughly 10 times more than Chromosorb W and the former has more H-bonding sites. Gillen and Scanlon (1972) showed that in the separation of menthol-menthone stereoisomers, all columns studied with liquid phases supported on Chromosorb P give significantly improved column characteristics relative to those prepared on Chromosorb W. The other benefit of using Chromosorb P is increased stability of the column. However, Ottenstein (1973) found that on Chromosorb P, oxygenated compound and amines tail badly, while hydrocarbons perform well. Chromosorb W is much less absorptive than Chromosorb P and is suited to handle oxygenated compounds and amines only after some additional treatment, but it is very friable and readily

fragments. The differences in adsorptive nature of the supports are due to surface area. Chromosorb P has 6-1/2 times the surface area per unit volume as Chromosorb W. In addition, their pH values differ: Chromosorb P has a value of 6.5 and Chromosorb W 8.5. The basic sites on Chromosorb W can cause tailing of acidic compounds such as aliphatic and aromatic carboxylic acids and phenols. Chromosorb P has acidic sites which can cause the tailing of basic compounds such as amines. Filbert and Hair (1969) found that the comparative increase in column efficiency is due to the higher surface area of the smaller-pore supports. The pore diameter ranges from 0.1 to 10.0 μ for P and from 2.0 to 20.0 μ for Chromosorb W. A thinner liquid film on the support with smaller pores results in an increase in column performance. In addition the greater surface area of the small pore support also enables effective distribution of the liquid phase at high loadings. Desty et al. (1958) suggested that Chromosorb P is in general a superior support, although nearly equivalent performance can be expected from Chromosorb W at smaller particle sizes.

The solid support is not inert and can interact with the oil constituents. The support effects include tailings and changes in retention times of the peaks (Ottenstein, 1963; Scholz and Brandt, 1962). In a few cases isomerization or dehydration (Ottenstein, 1963) and inversion of elution sequences (Scholz and Brandt, 1962) have also been observed. To reduce significantly the double bond isomerization of the terpenes Klouwen and Heide (1962) suggested the use of acid washed grade support. Ottenstein (1963, 1973) analyzed the chemical composition of solid supports before and after acid treatment and found that acid washed Chromosorb P and W have lower contents of aluminum, iron, calcium and

alkali metal oxides due to the removal of impurities from the support surface. Similarly, Karger and Cooke (1964b) found that acid washing removes metal groups which may act as adsorption sites.

The solid supports are partly deactivated by liquid phases (Scholz and Brandt, 1962; Ottenstein, 1963, 1973). The degree of deactivation depends on the extent to which the functional group of the liquid phase can form hydrogen bond with the silanol groups (Ottenstein, 1963, 1973). Liquid phases containing OH or primary amine groups are very effective, while those with carbonyl or ether groups are less effective.

The particle size of the support affects the theoretical plate height of the column. As the size of the particle is reduced, the column efficiency is increased. Thus an 80 to 100 mesh column exhibits three times the efficiency of a 30 to 35 mesh column (Nogare and Juvet, 1966). Karger and Cooke (1964b) suggested that the resolution decreases with larger particles due to a capacity factor which decreases as the particle size increases. In addition, Nogare and Juvet (1966) found that the pressure drop increases roughly proportional to $1/d_p^2$ where d represents the effective diameter of the particle. Hence, a column packed with support consisting of small particles is efficient when operated at elevated pressure.

3. Liquid Phase

Many of the liquid phases have been used in analyses of essential oils. The phases used include glycol derivatives, esters, hydrocarbons and silicons in decreasing order of preference, Of the glycol derivatives Carbowax 20M is preferred. Diacetate hexaisobutyrate (SAIB) among the ester class and Apiezon L among the hydrocarbon class of liquid phases

are mostly preferred. Of the silicone liquid phases, Silicone DC-550 is widely used.

Zubyk and Conner (1960) established that didecyl phthalate is a non-selective liquid phase for the separation of terpene hydrocarbons which will be eluted in the order of their boiling points. Carbowax 4000 shows some selective retention for unsaturated compounds with conjugated double bonds. Furthermore, they found that other liquid phases such as mineral oil, tritolyl phosphate, Apiezon J and N, ethyl tetrahydroadiebate, dihydroabietyl alcohol and an alkyl phenol-ethylene oxide adduct had no significant advantages over those two liquid phases. However, some samples of didecyl phthalate were found to possess sufficient acidity to isomerize α- and β-pinene. Von Rudloff (1960) reported that polar packings such as ethylene glycol polyesters of succinic and phthalic acids give a separation of monoterpene hydrocarbons comparable to that of Carbowax 4000 and polyethylene glycol adipate. These liquid phases also efficiently separated the oxygenated terpenes because they are stable at temperature above 130°. In addition, squalene produced good resolution of monoterpene hydrocarbons and was found to be stable above 130°. Nonpolar and slightly polar liquid phases such as silicon oil, didecyl phthalate, poly (dimethyl siloxane) and Apiezons are other phases used to separate monoterpene hydrocarbons. As indicated by Burchfield and Storrs (1962) these separations are based on differences in the boiling points of these compounds.

Oxygenated terpenes, especially those of the monoterpenoid class, were successfully separated on polar stationary phases which are stable between 130° and 160°. Among these phases are the polyesters of adipate and polyethylene glycol, and of succinate and ethylene glycol (Von Rudloff,

1960; Burchfield and Storrs, 1962). The succinate polyester of diethylene glucol and adipate polyester partially crosslinked with pentaerythritol also produced good separation of oxygenated terpenes (Bernhard and Marr, 1960). Oxygenated monoterpenes were also well separated by the liquid phase of SAIB which was stable up to 200° (Smith et al., 1960). The Apiezons and silicone oils are among the relatively nonpolar liquid phases which were also used to separate oxygenated monoterpenes such as linalool, nerol and geraniol (Cartoni and Liberti, 1960; Popjak and Cornforth, 1960). However, Datta and Susi (1962) were able to elute oxygenated monoterpenes in the order of their boiling points with the nonpolar phase, Dow Corning 710, but not on a polar phase consisting of polyethylene glycol adipate. Similarly, Von Rudloff (1960) had shown that α- and β-eudesmol are only partially separated on polyethylene glycol adipate and ethylene glycol succinate but not on silicone oil used as a nonpolar phase. The menthol isomers are also well separated on silicone oil, and with di-n-decylphthalate as a polar phase separation has been achieved for three of these isomers (Petrowitz et al., 1960). Similarly, at a column temperature of 160°, three menthol stereoisomers have been separated on Carbowax 4000 (Tagaki and Mitsui, 1960). Among several liquid phases evaluated Carbowax 4000 on Chromosorb P gave the best overall separation for menthol-menthone stereoisomers (Gillen and Scanlon, 1972). Contrary to this, alcohol-amine phases such as Teed and Quadrol showed a tendency of coalescing and broadening indicating an on-column reaction which, as found by Van Swaay (1969), involves mostly enolization. Generally, polar liquid phases give better separations of aliphatic and cyclic monoterpene alcohols. These liquid

phases also give good separations of aldehydes and ketones. Furthermore, as found by Cartoni and Liberti (1960), linalyl and geranyl acetates can also be separated by these phases.

Breckler and Betts (1970) studied the relative retention time changes of some essential oil constituents with temperature using three stationary phases; silicone oil (SE-30), a nonpolar phase, diethylene glycol succinate-polymer (DEGS), a slightly polar electron-donor phase, and Carbowax 20M a more polar electron-acceptor liquid phase. For most of the slightly polar substances such as citral or anethole, the retention times relative to linalool are greatest on DEGS, showing that their polarity is best matched by the DEGS polarity. Most of the terpenoid alcohols had the greatest retention times on Carbowax 20M, while the relative retention times of terpenoid carbonyls and substituted aromatic compounds were greater on DEGS than on Carbowax 20M. From the data on relative retention times it was also concluded that the nonpolar column SE-30 is best suited for terpene hydrocarbons and 1,8-cineol. Finally, the authors concluded that according to the polarity match principle, terpene hydrocarbon and ester containing oils should be chromatographed on nonpolar columns, terpene alcohol containing oils on polar columns, and oils with terpene carbonyls or aromatics on slightly polar ester columns. However, as the essential oils are mostly complex mixtures, one type of column is unlikely to produce the best overall separation of all the components. Because of the above finding, Humphrey (1970) suggested the use of nonpolar columns for nonpolar oils and vice versa. Thus, Apiezon L and methyl- or phenyl silicone oils were suitable for pepper, ginger and celery oils, but were less suitable for oils containing large quantities of oxygenated constituents, particularly alcohols, due to excessive

tailing on pure Apiezon L. The tailing could be eliminated by adding a small quantity of free fatty acids to the Apiezon L without affecting either the order of elution or the retention time. However, free fatty acids have a deleterious effect on aldehyde constituents at temperatures above 150°. Among the polar columns, Carbowax 20M, which has intermediate polarity, gave good separation for all essential oils. In addition, it was found to be thermally stable up to 250°, not prone to ester interchange reactions and constant in performance. When Carbowax 20M is used at 250° the least volatile oil constituent will readily be eluted.

4. Liquid Phase Loading

The amount of liquid phase loading also can affect the retention time, and separation efficiency of the column. Martin (1961) observed that the elution order from columns containing polar liquid phases varies with the ratio of liquid phase to solid support, with the surface area of the support and, to a lesser extent, with temperature. At lower liquid loadings the operation can be carried out at a lower temperature without losing the separating power of the column (Frederick et al., 1962). However, Sawyer and Barr (1962) observed that in many cases a low loaded siliconized Chromosorb W column is more efficient than a siliconized Chromosorb P column. Frederick et al. (1962) were able to prepare columns of high efficiency with a lightly loaded liquid phase on glass beads and Chromosorb P. They also found that, under the conditions studied, the efficiency of a Chromosorb W column deteriorates as the amount of liquid phase decreases.

Scholz and Brandt (1962) observed that the retention volume increases almost linearly with the amount of liquid phase for all solutes

except alcohols. Ottenstein (1963) and Humphrey (1970) found that there is no such linear relationship between the amount of liquid loading and retention volume. The increase in retention becomes less as the loading increases. On the other hand, the adsorptive effect of solid support depends on the amount of liquid phase. As the amount of the phase is decreased the adsorption effect becomes more noticeable. This effect is greater with Chromosorb P than Chromosorb W when relatively nonpolar liquid phases are used (Ottenstein, 1963). Thus, Chromosorb P, which has a smaller pore diameter range (0.1 to 10.0 μ) and a larger surface area, has a more effective distribution at high loadings (Filbert and Hair, 1969). It was also observed that high loadings give good separation efficiency and an increase in column overall capacity. As the amount of loading increased up to 15%, the tailing of the peaks was reduced. Further increase of loading had detrimental effect on column performance (Ottenstein, 1973).

5. Column Conditions

Gerrard et al. (1960) and Keller et al. (1962) have shown that heating at a high temperature for a prolonged period tends to degrade the stationary phase and to increase the phase volatility. As a result, there is a change in the performance of the column. Chen and Gacke (1964) observed that high temperature causes a loss in polarity of the column and that this loss is more pronounced for polar than nonpolar stationary phases. Apiezon L, a relatively nonpolar stationary phase, develops a slight polarity due to a certain degree of oxidation during its use (Humphrey, 1970). On the other hand, no deterioration of performance of Carbowax 400 was found when the column was used for weeks at

25° above the recommended temperature of 125° (Gillen and Scanlon, 1972). Generally, chemical and physical changes of the liquid phases are most marked at the inlet end of the column (Keller et al., 1962). This is due to the high temperature of the injection port and to the injection of impurities and other reactive substances into the column. As stated by the same authors, chemical changes in the liquid phase can be caused by impurities of the carrier gas, particularly oxygen, non-volatile impurities in the partitioning liquid such as the H^+ ion, catalytic reaction with the support, catalytic reaction with degradation products arising from the liquid phase, and by a further condensation of the polymeric liquid phase.

Martin (1961, 1963) observed that as the amount of liquid phase changes, the elution order from a column might also change. This was ascribed to the adsorption of the constituents both on the liquid phase and solid support. The effect was more pronounced when the liquid phase was highly polar, surface area of the support was high, and when liquid phase loading and the temperature were low.

6. Sample Size

Scholz and Brandt (1962) showed that sample size affects the retention volume but has little or no effect when either Chromosorb W coated with a liquid phase or Chromosorb P coated with Carbowax 400 are used. In addition large sample size was found to broaden individual peaks and increase retention time (Nogare and Juvet, 1966). The sample size to be injected also depends on the size of the column. VandenHeuvel and Kuron (1969) have shown that there is no difference in column performance between narrow (I.D. 4 mm) and wide columns (I.D. 11 mm) when

small quantities of sample are analyzed. With larger quantities the performance of the wide columns is superior. Increase in sample size caused a considerable decrease in the observed theoretical plate value. With a 100 µg injection the smaller columns were overloaded as shown by distorted peak geometry. This overloading was also observed in wider columns when a 500 µg sample was used.

Recently, Harris (1973) also demonstrated that even with thorough control of the column conditions, the retention times vary with large sample size. The maximum sample volume recommended varied with different compounds and it generally increased with the components with higher retention times. The sample size also depended on the initial but noton the programmed temperature. Thus, a larger sample size could be injected when the initial temperature was low. However, when a larger sample is used and the inlet temperature of the column is low, the higher hydrocarbons are retained so strongly at the inlet that broadening of peaks can occur. Also, with the large sample, the major components tend to influence the movement of the minor components near to them. Those constituents that are more strongly retained than the major component are more affected than those that are less strongly retained. This results in a chromatogram with peaks tending to group together more closely in the region of a major component, hence, the resolution of these peaks will be poorer..

7. Injection Temperature

Injection temperature is important because it should be high enough to evaporate the essential oil constituents immediately following the sample injection and not high enough to cause thermal decomposition

of the constituents. Day and Miller (1962) found that an injection temperature of 205° caused decomposition of α-terpineol and linalool, but no decomposition was observed at 100°. However, other studies showed that injection temperatures of 216° and 275° (Datta and Susi, 1962; Mitzner, 1964) do not cause decomposition of α-terpineol. Therefore, the decomposition of oil constituents can be attributed to overheating of the injection port end of the column, to inaccurate temperature recording, to an abnormal hot spot in the injector and, finally, to a possible accumulation of acidic compounds in the injector port (Mitzner, 1964). In agreement with the latter, Day and Miller (1964) established that the decomposition of α-terpineol could have been due to the organic acids present in the flavor concentrate. In addition, as found by Mitzner (1964), compounds such as organic chlorides and conjugated tertiary esters are thermally unstable.

8. Temperature Programming

The essential oils contain many constituents with a wide range of boiling points; in peppermint oil the boiling point of α-pinene is 157° and of piperitone 233°; in anise oil the boiling point of eugenol is 253°. Thus in the analysis of these essential oils it is necessary to separate the constituents under temperature programming. When the boiling point of the oil constituent is lower than the temperature of isothermal chromatography, the constituent is eluted quickly with a narrow peak which can not be measured precisely and there is also a serious loss of resolution. On the other hand, the component with a boiling point higher than column temperature is eluted after a longer time with a broad and low peak which can be measured with low precision and with a

poor detection limit. In temperature programming the lower boiling point components will emerge earlier as if the column is operated at relatively low isothermal temperature and higher boiling components will be bunched up by the increasing temperature and will emerge with sharper peaks. As a result an extremely wide boiling range of components can be separated in less time, with sharper and more uniform shaped peaks.

Harris and Habgood (1966) suggested that temperature programming is advantageous when the boiling point range of the constituents is 50°, and that the usefulness of such programming increases as the boiling range is greater. Further, by comparing the precision of peak area measurement in isothermal and temperature programming chromatography, they found that the precision varies with retention time in isothermal but is constant in temperature programming chromatography.

The initial temperature should be based on the vapor pressures of the most volatile components in the sample. For a packed column, the initial temperature may not be much below the boiling point of the most volatile component (Harris and Habgood, 1966). In general, the starting temperature is between 50° and 75° (Humphrey, 1970). The terminal temperature in a packed column should be approximately equal to the boiling point of the least volatile component, but other factors, such as temperature stability of the stationary liquid phase of the components themselves, may determine this upper limit (Harris and Habgood, 1966).

The heating rate should be inversely proportional to the total number of constituents (Harris and Habgood, 1966). From their chart of frequency of heating rate used in Programmed Temperature Gas Chromatography up to the end of 1963, it was concluded that the rate 1° to 3°/min,

3° to 5°/min and 5° to 10°/min were most frequently used. The average value was a rate of 3.5°/min, and rates of more than 10°/min were rarely applied. For analysis of essential oils Humphrey (1970) reported that the choice of program rate should be governed by the type of resolution required and that it lies between 1° and 5°/min.

9. Coupling of Gas-Liquid Chromatography - Mass Spectrometer for Qualitative Analysis

Perry (1967) stated that GLC - Mass Spectrometer analysis provides more specific structural information about organic molecules than any other single technique and gives excellent mass spectra with only microgram quantities of pure substance. As Teranishi et al. (1971) pointed out, the principal advantage of the GLC - Mass Spectrometer technique is its convenience. There is no loss or contamination of sample as usually happens with the trapping of emerging peaks. The component separated by GLC enters into the Mass Spectrometer ion source compartment at its maximum purity. Many spectra can be obtained from individual peaks as they emerge from the column. The information obtained from spectra scanned at the beginning, middle and end of the peak helps in the detection and identification of incompletely resolved components.

One problem which arises from this technique is the presence of column bleed in trapped gas chromatographic fractions (Perry, 1967; Teranishi et al., 1971). The longer the trapping time, the greater will be the contamination of the sample by the liquid phase. The higher the temperature in the temperature programmed GLC, the greater will be the bleeding of the column. Therefore, the same authors suggested the use of less volatile silicones to reduce this bleeding.

10. Identification of the Components

A comprehensive review of methods for identification of components separated by GLC is given by Perry (1967). Peak identification can be done purely by gas chromatography using retention time, prediction of retention time and by a multi-column system. The peak identification can also be carried out by chemical modifications of the sample such as in a subtractive method, peak shifting techniques, catalytic conversion, pyrolysis and other chemical methods, e.g., coulometric reactions or the appearance of a precipitate or color. The identities of the peaks can be further elucidated by auxillary qualitative detectors such as used in ultraviolet spectrometry, infrared spectrometry and mass spectrometry. Arakelyan and Sakodynskii (1971) reviewed the methods of peak identification using only gas chromatography. They emphasized that the comparison of retention times obtained with one column under similar conditions only allows the identification of already known substances.

III. MATERIALS AND METHODS

Herbs and Oils

The essential oil samples distilled at the Alberta Horticultural Research Centre, Brooks, were anise seed oil--1971 and 1972 crops, caraway seed oil--1971 crop, dill seed oil (two common dill samples and one Danish dill sample)--1971 crop, fennel seed oil--1971 and 1972 crops, peppermint oil (black or English mint)--1970 crop (harvested at five stages of growth: bud stage, beginning of blooming, 75% blooming, full blooming and end of blooming), and sage oil--1971 and 1972 crops. Two samples were obtained from Beaverlodge, Alberta. The quality of Alberta oils was compared with that of some world market oils, namely anise seed, caraway seed, standard and prime dill herb and sage oils from Kalsec Int., Kalamazoo, Michigan, fennel oil from Fritzsche, New York, and Michigan peppermint oil from Hotchkiss, Lyons, New York.

The data for Brooks on harvesting, sample preparation, distillation and yield of the 1970 peppermint herb trial are given in Table 1, herbs and spices 1971 trials in Table 2, and herbs and spices 1972 trials in Table 3. The weather data for 1971 are given in Table 4 and for 1972 in Table 5. The data on soil analyses are given in Table 6.

Chemicals

Anise acetone ($\geq$ 99.0%), anisic aldehyde (ex anethole, $\geq$98.0%, n_D^{20} 1.571-1.573), borneol (m.p. 200°-204°), bornyl acetate (crystal, laevo $\geq$98.0%, n_D^{20} 1.461-1.464), D-camphor (DAB VII, $\geq$96.0%, m.p. 170°), D-carvone (pure from caraway oil, $\geq$95.0%, n_D^{20} 1.496-1.500, $[\alpha]_D^{20}$ +58 to +60), 1,8-cineol (eucalyptol, $\geq$97.5%, n_D^{20} 1.455-1.460), eugenol (ex

TABLE 1

PEPPERMINT HERB - 1970 TRIAL

	Stage	Harvest Date (1970)	Distilled Date (1970)*	Oil Yield %	Green Plant Weight
1.	Stage I (bud stage)	Aug. 1	Aug. 18	0.204	101 lb/280 ft^2 plot
2.	Stage II (beginning of blooming)	Aug. 8		0.215	
3.	Stage III (75% blooming)	Aug. 22	Sept. 20	0.186	
4.	Stage IV (full blooming)	Aug. 29	Sept. 26	0.183	
5.	Stage V (end of blooming)	Sept. 12	Sept. 20	0.180	

*Whole plants are dried, crushed and distilled by steam in a distillation unit simulating the large scale portable units. The oil obtained was separated from water in a laboratory separatory funnel and dried with Na_2SO_4 and the weight was reported as oil yield. For further chemical analyses the oil was stored at 0-4°C.

TABLE 2

HERBS AND SPICES - 1971 TRIALS

Plant	Sample Preparation*	Dry Weight of Material Distilled (g)	Oil Yield %
Anise	Most of the seeds were mature green. Whole plant dried, crushed and distilled.	469	1.05
Caraway	Around 50% of seeds mature. Only the seeds were dried and distilled.	225	2.65
Danish dill	Around 50% of seeds mature. Only the seeds were dried and distilled.	496	1.18
Common dill	Same as Danish dill.	621	1.82
Fennel	Plants mature when harvested. Whole plant dried, crushed and distilled.	345	1.44
Sage	70% blooming at harvest. Whole plants dried, crushed and distilled.	2390	0.25

*All plants harvested Sept. 13. Steam distillation and storage of oils as in Table 1.

TABLE 3

HERBS AND SPICES - 1972 TRIALS

Plant	Harvest Date	Sample Preparation*	Yield
Anise	Sept. 26	Only seed harvested. Seed thoroughly ground in Wiley mill.	adequate
Dill		Entire plant harvested. Air dried at temperature approximately 15° to 21° for 2 to 3 weeks.	low oil yield
Fennel		Seed harvested. Seed dried same as dill.	
Sage	Aug. 28	Entire plant harvested. No drying.	no oil obtained
	Aug. 29	Entire plant harvested. No drying.	no oil obtained
	Sept. 1	Entire plant harvested. Drying at 22° for 3 days.	good oil yield

*Steam distillation and storage of oils as in Table 1.

TABLE 4

WEATHER DATA - 1971

Month	No. of Days	Precip.	Hours of Sunshine	Frost Free Period	Remarks
May 16-31	16	1.21	142.5	16	Cool temperatures 43-65°F(day), 33-42°F(night) with 1.30" precipitation from 15-20th, improving to 80°'s by 25th. On 27th some thunder activity with hail in some areas. Soil conditions cool and wet, delay in some cultivation and planting.
June	30	1.53	296.9	30	Normal day temperatures accompanied by cool nights. Wind and shower activity 4,9,17&18th. Thunder showers with hail in some district areas. Very cool, nights from 26-30th down to 37°F. Periods of strong winds during the month.
July	31	0.79	362.5	31	Cool day and night temperatures,2-14th moderate precipitation. Latter half temperatures improved. Sunny, hot and dry. Very strong winds on 23rd, gusting over 40 mph S.W.

(Continued)

Table 4, Page 2 (continued)

Weather Data - 1971

Month	No. of Days	Precip.	Hours of Sunshine	Frost Free Period	Remarks
August	31	1.40	362.4	31	In general, a hot month, mercury reaching 97°F and on many recordings 90°F or more. Very little precipitation up to 23rd when 1.22 inches were recorded. Winds light.
September	20	0.59	137.9	17	1-14th temperatures good both day and night, light showers. Cooling trend began on 15th accompanied by heavy showers. Strong winds recorded on 11th.
Totals	128	5.52	1302.2	125	

Total Precipitation for year - 12.06

Summary of Growing Period - May 16 to September 30

- first half cool day and night temperatures, light periodic precipitation, some strong winds. Latter half sunny, hot and mostly dry, with light periods of rain, some hail in district areas

TABLE 5

WEATHER DATA - 1972

Month	No. of Days	Precip.	Hours of Sunshine	Frost Free Period	Remarks
May 8-13	24	1.06	218.7	19	May came in dry, 12°frost 6&7th. Cool temperatures prevailed until the 12th when 82° was recorded on the 13th. Strong winds from 15-22nd. Thunder activity and showers. Warm temperatures and rain from 22-27th. Remainder of month hot--up to 87°.
June	30	2.10	313.3	30	First week hot and dry, light showers from 9-14th, on 11th a 24 h period of strong S.W. winds, cool temperatures on 19th, some frost in local areas, remainder of month average, light shower activity, with periods of strong S.W. and S.E. winds.
July	31	1.48	274.1	31	Month came in with cool strong northerly winds, very dry conditions, 14-31st shower activity, daily temperatures 6° below normal and sunshine 2.6 hr below daily average for 15-year period.

(Continued)

Month	No. of Days	Precip.	Hours of Sunshine	Frost Free Period	Remarks
August	31	0.51	330.6	31	Typical summer weather first week, showers on 8,9&10th totalling 1/2" Remainder of month hot and dry with periods of strong northerly winds.
September 1-20	20	1.55	126.9	6	First 5 days average fall weather, 6-10th temperatures dropped to 42° day and 32° night with mixed snow and rain, strong north winds, similar conditions 15-20th and 23-27th, remainder of month warm.
Totals	136	6.70	1263.6	117	

Summary of Growth Period - May 12 to September 6

- rather cool summer with isolated "few dry periods" of normal high temperatures, average precipitation. August brought in seasonal temperatures with dry conditions, followed by periods of cool temperatures with snow and rain in September

Note: Average sunshine hours for July, 15-year period 353.9 hours

TABLE 6

SOIL ANALYSIS DATA

Sample	Pounds Per Acre Available Nutrients				Soil Reaction (pH)	Conductivity* (mmho.)	Sulfates (ppm)	Estimated	
	Nitrogen (N)	Phosphorus (P)	Potassium (K)	Na				Organic Matter	Free Lime
1	24	111	670	L†	7.9	0.4	3.8	L+	L 2
2	25	62	505	L	8.1	0.7	74.6	L+	L+ 2
3	33	34	410	L+	7.9	1.0	106.0	L	L 2

*conductivity, 0-2 negligible salt effects
†L, low

Clove oil, n_D^{20} 1.540-1.542), limonene (pure, n_D^{20} 1.471-1.474, $[\alpha]_D^{20}$ +96 to +104), linalool (≥99.0%, n_D^{20} 1.460-1.465), linalyl acetate (≥98.0%, n_D^{20} 1.448-1.454), α-pinene (n_D^{20} 1.462-1.470) and β-pinene (n_D^{20} 1.472-1.480, $[\alpha]_D^{20}$ -14 to -21) were supplied by Haarmann & Reimer Gmbh., Holzminden, W. Germany. 4-Allylanisol (estragole, ≥98.0%, n_D^{20} 1.5193), trans-anethole purum, USP (n_D^{20} 1.5614, m.p. 21°-23°), anisyl alcohol (m.p. 23°-25.5°, n_D^{20} 1.54420), p-anisic acid (zone refined, ≥99.9%, m.p. 183.05°), L-borneol (m.p. 208°, $[\alpha]_D^{20}$ -35.3), camphene (80.0% m.p. 44°-48°), p-cresol (99.0%, m.p. 32°-34°), p-cymene (99.0%, m.p. 73°, n_D^{20} 1.4897), dipentene (tech., n_D^{20} 1.4739), farnesol (≥95.0%, n_D^{20} 1.4889), menthol (99.0%, m.p. 28°-30°), L-menthol (m.p. 43°-45° $[\alpha]_D^{20}$ -50), menthone (n_D^{20} 1.4510), myrcene (tech., n_D^{20} 1.4715), and vanillin (99.0%, m.p. 81°-83°) were obtained from Aldrich Chem. Co. Inc., Milwaukee, Wisconsin.

(-)-Fenchone (pract.≈95.0%), (+)-fenchone (purum ≥98.0%, n_D^{20} 1.463, $[\alpha]_{546}^{20}$ +75±3), (-)-limonene (98.0%, $[\alpha]_{546}^{20}$ -135 5), 3-octanol (purum 97%, n_D^{20} 1.424), α-phellandrene (tech., 50.0% α-phellandrene, 12-15% β-phellandrene, 15-20% p-cymol), (+)-pulegone (purum 96.0% $[\alpha]_{546}^{20}$ +29 1) and α-, β-thujone (tech.) were from Fluka AG., Buchs, Switzerland. Apiol (95-99%), iso-borneol (95-99%), β-caryophyllene (tech.≈90.0%), iso-bornyl acetate (95-99%), dihydrocarveol (95-99%), dihydrocarveyl acetate (95-99%), isomenthone (95-99%), DL-menthone (95-99%), menthyl acetate (95-99%), α-phellandrene (95-99%), piperitone (95-99%), and α-terpineol (95-99%) were obtained from K & K Labs. Inc. Plainview, New York. Menthofuran (b.p. 86°-87°/15 mm), and 2', 7'-dichlorofluorescein (≥98.0%) were from Eastman Org. Chem. Rochester, New York. Vanillin (refined - U.S.P.) was obtained from Merck (Merck AG. Darmstadt, Germany).

Column packing, 15% EGS (ethylene glycol succinate polymer) on Chromosorb P, AW, 100/120 mesh, was from Chromatographic Specialties, Brockville, Ontario. Kieselgel N, without binder, was obtained from Macherey Nagel & Co., Düren, W. Germany.

Isomenthol and neoisomenthol were synthesized by us from isomenthone, carveol from carvone, and neomenthol from menthone, using aluminum isopropoxide and applying the Meerwein - Ponndorf - Verley reduction procedure. Neomenthyl acetate, neoisomenthyl acetate and terpinyl acetate were also prepared by us, by refluxing neomenthol, neoisomenthol and terpineol respectively in xylene with glacial acetic acid, acetic anhydride, and fused sodium acetate.

Syntheses of Neomenthol, Neoisomenthol and Carveol by Meerwein - Ponndorf - Verley Reaction

A solution of 20 g aluminum isopropoxide in 100 ml dry isopropyl alcohol was placed in a 250 ml round bottomed flask, then 15 ml of the ketone (menthone, isomenthone or carvone) was added, and the flask was fitted with a modified Hahn (air-type) condenser. The mixture was heated to a distillate drop rate of 5 to 10 drops/min. When two successive tests, about 10 min apart, gave no precipitation with 2,4-dinitrophenylhydrazine reagent within 30 seconds, the run was stopped. The bulk of the isopropyl alcohol was removed under reduced pressure. The residue was cooled in ice, and cold dilute HCl (35 ml HCl:175 ml H_2O) was added slowly, shaking the flask content. The alcohol obtained was extracted with benzene, the extract was then washed with a further portion of the cold acid, then with water, and finally dried over anhydrous Na_2SO_4. The bulk of the benzene was distilled off, and the alcohol was collected.

Syntheses of Neomenthyl-, Neoisomenthyl- and Terpinyl-acetates

A solution of 5 g of the alcohol and 100 ml of xylene was placed in a 250 ml round bottomed flask; 40 g acetic anhydride and 3 g of fused anhydrous sodium acetate were added and the mixture was refluxed for about 6 h. After cooling, 50 ml of distilled water was added and the mixture was additionally heated for 30 min. The water layer was removed, and the xylene-acetate mixture was washed once with 50 ml of hot distilled water, and then dried over anhydrous sodium sulphate. The solution was filtered, and the xylene was distilled off. The residue (the acetate) was collected.

Equipment

Thin-layer chromatography equipment used was from Shandon Scientific Co., Ltd., London, U.K. Infrared spectra were recorded by using: 1) a model 21 spectrophotometer, The Perkin-Elmer Corp., Norwalk, Connecticut, and 2) an I.R. 20 Infrared spectrophotometer, Beckman Instrument Inc., California. The KBr pellets were made with RIIK equipment, Research Industrial Instrument Co., London, U.K. The centrifuge was a model SS-1 Superspeed Angle Centrifuge, Ivan Sorvall Co., Inc., Newtown, Connecticut. Gas chromatography equipment used was: 1) a model 2500, Bendix Instruments Div., Ronceverte, W.Va., and 2) a Series 1400, Varian Aerograph, Walnut Creek, California. The Varian Aerograph, Series 1400 was coupled with an MS 12 mass spectrometer, produced by Associated Electrical Industry, Manchester, U.K. The preparative plates were viewed with a short-wave ultra-violet 'minerallight' model SL 2537, Ultra-Violet Product Inc., South Passadina, California.

Methods

A. Thin-Layer Chromatography

A slurry of 25 g of MN-Kieselgel N (without binder) and 60 ml of water was spread on five 20 x 20 cm plates, at a thickness of 300 μ. The plates were dried at room temperature for 1 h, and then activated at 110° for 1-1/2 h. After cooling to room temperature in a dessicator the plates were ready for use.

1. Qualitative Thin-Layer Chromatography

The essential oil and its associated standards were spotted 1-1/2 cm from the bottom of the plates. The essential oil and standards in liquid form were spotted neat, while solid standards were dissolved at high concentration in ethyl acetate. The plates were developed for about 40 min with a solvent system of benzene:ethyl acetate (95:5 v/v).

Spots were visualized by spraying with a solution of 1% vanillin in concentrated sulphuric acid and then heating at 105° for 10 min. The Rf values and the colors of the spots from essential oils were used to determine the identity of the components.

2. Preparative Thin-Layer Chromatography

A similar procedure as described above was followed, but in this preparative TLC, a strip of neat essential oil was spread on the plate. After visualization, the bands of the components were traced on transparent paper for future localization of the bands. In addition, several plates were sprayed with 0.5% of 2,7-dichlorofluorescein in ethyl acetate saturated with water. The bands were viewed under UV light. Not all the bands could be seen under such light, but with the help of the tracing

made earlier, all the bands could be allocated. The bands were then scraped from the plate and the components from the adsorbent were extracted by vigorous shaking in ethyl acetate. The mixture was then centrifuged at 7,000 x g for 10 min and the supernatant obtained was decanted and concentrated in a stream of pure N_2 (purity 99.8%). Two or three preparative plates were necessary to obtain sufficient quantity of the components for further analysis. The identity of the components was established by coinjection into a GLC with 0.1 µl of the original sample of essential oil.

B. Gas-Liquid Chromatography

1. Quantitative Gas-Liquid Chromatography

A volume of 1 µl of the essential oil was injected into a GLC fitted with two 6 ft x 1/8" I.D. glass columns packed with 15% of EGS polymer on Chromosorb P, AW, with particle size of 100/120 mesh. The operating conditions for peppermint oil were: injection temperature 220°, detector temperature 240°, N_2 carrier gas flow rate 30 ml/min, column temperature programmed from 60° to 150° at 1°/min, Amps full scale sensitivity 2 x 10^{-8} and chart speed 20 cm/h. The operating conditions for anise, caraway, dill, fennel and sage oils were as above except that the N_2 gas flow rate was 60 ml/min, column temperature programmed from 50° to 195° at 4°/min and chart speed 60 cm/h. For anise and sage oils it was necessary to hold the maximum temperature for 15 to 30 min to get the final peak. Quantitation of the individual peaks was done by triangulation technique with an accuracy equal or better than 0.5%.

2. Qualitative Gas-Liquid Chromatography

Each standard compound was dissolved in ethyl acetate and co-

injected with 0.1 µl of the essential oil on the 'fresh' and 'aged' columns. The fresh columns were those which were freshly prepared while the aged columns were those which had been used for quite some time.

C. Infrared Spectral Analysis

The IR spectra of the essential oils and reference compounds in liquid form were recorded neat between two NaCl plates. The operating conditions of the P-E model 21 were: resolution 927, response 2, gain 6, speed 4, auto suppression 0, filter auto, and scale 5000 to 650 cm^{-1}. The spectra for solid compounds were obtained from the Beckman 20 instrument using KBr pellets, containing about 10% of the component. The operating conditions were: Scan speed 240 cm^{-1}/min, period 2, gain 5, balance 0, and scale 4000 to 600 cm^{-1}.

D. Mass Spectral Analysis

A volume of 0.3 µl of the essential oil was injected into a Varian 1400 GLC fitted with a coiled stainless steel column 6 ft x 3/16" I.D., packed with 15% EGS polymer on Chromosorb P, AW, 100/120 mesh. This GLC was coupled with an MS 12 mass spectrometer. The major peaks were scanned. The GLC operating conditions were: He carrier gas flow rate 70 ml/min, chart speed 1 cm/min, and column temperature programmed from 50° to 195° at 4°/min. Mass spectrometer operating conditions were: magnetic coil 2, Ws (width of the source slit) 0.004", Wc (width of collection) 0.004", magnetic range 5, decrease speed 10, band width 1000 H_z, chart speed 6"/sec, trap current 100 µA and multiplier 310. The liquid standards were run under similar conditions, while solid standards were run by direct probe with electron voltage of 70 and source temperature of 115°.

IV. RESULTS AND DISCUSSION

A. Thin-Layer Chromatography - General Observations

The overall TLC separation of the essential oil components based on the functional groups is illustrated in Fig. 1. Most of the spots of essential oil components with hydroxyl (OH) groups were found between Rf values of 0.12 and 0.29; carbonyl (CO) groups between Rf values of 0.29 and 0.47; acetate ($OCOCH_3$) group between Rf values of 0.47 and 0.59; ether (COC) groups between Rf values of 0.59 and 0.66; and hydrocarbons between Rf values of 0.66 and 0.73. The Rf values between 0.00 and 0.12 were most probably the region of the components having carboxyl (COOH) or polyhydroxyl groups. For instance, the Rf value of p-anisic acid was 0.02.

With the solvent system and adsorbent used in this study, the adsorption affinity of the functional groups increased in the following sequences, CH_3, COC, $OCOCH_3$, CO, OH and COOH. This is in accordance with the results obtained by Brockmann and Volpers (1949) and Stahl (1969). The sequence of the TLC separation depends on the polarity of the functional groups. The difference in Rf values of constituents having the same functional group is due to the difference in the configuration, the presence and the number of double bonds. Among the oil constituents having OH groups, borneol was found to be more polar than linalool. Borneol is a secondary alcohol whereas linalool is a tertiary alcohol. In addition, the OH group in linalool is adjacent to a methyl group which imparts a steric hindrance to the polarity of the OH group.

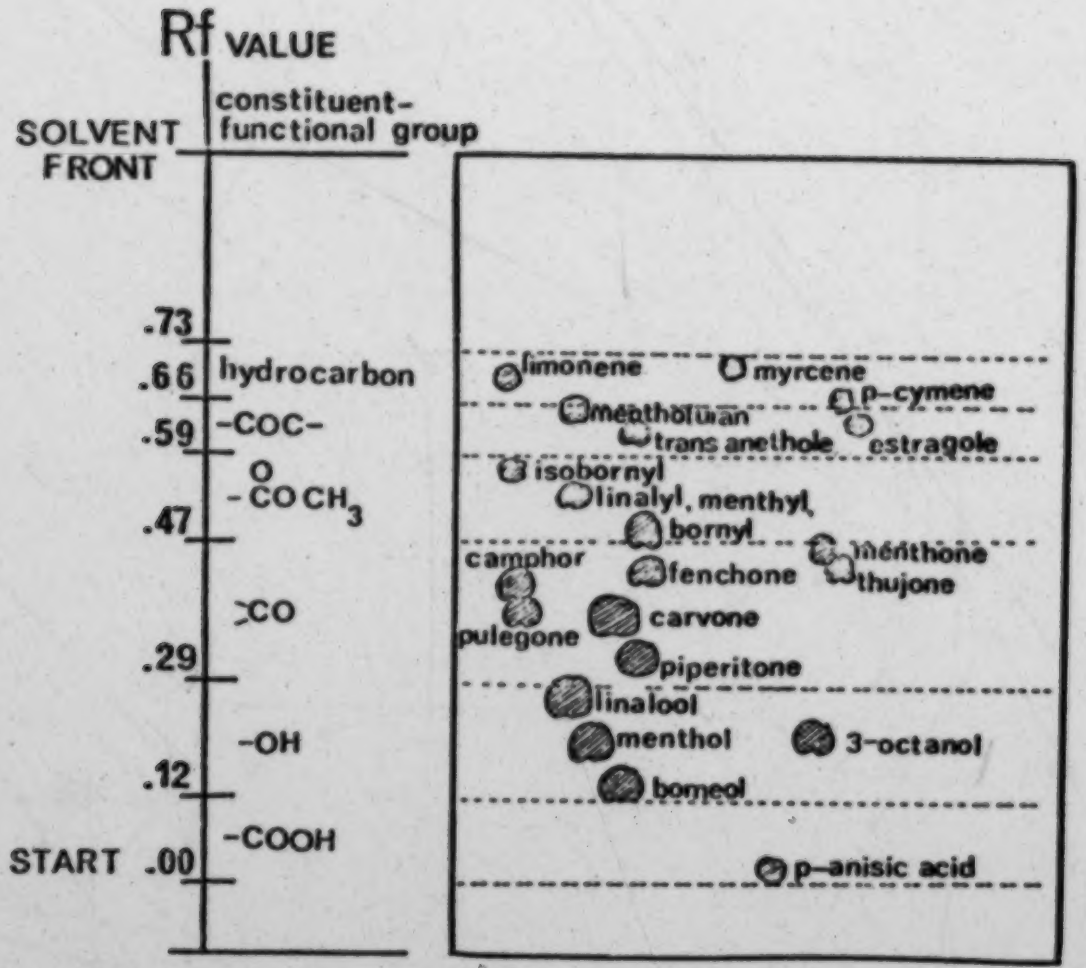
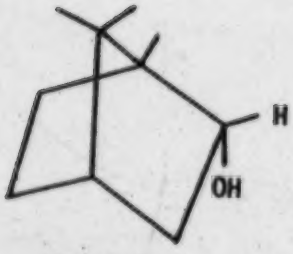


Fig. 1. Relationship of Rf Values and Functional Groups of Essential Oil Constituents.

Adsorbent layer: 300 µ of MN-Kieselgel N (Macherey Nagel & Co)
Solvent system: benzene-ethylacetate, 95:5 v/v.
Spot detection: spraying 1% vanillin in conc. sulfuric acid and heating in oven at 105° for 10 min.
Volume of pure oil applied: 1 µl.

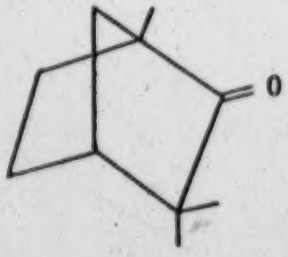


Borneol

Linalool

Of the ketone constituents, piperitone was found to be more polar than fenchone. Piperitone has a carbonyl group and a double bond conjugated with CO whereas fenchone has only CO group. Further, the CO

Piperitone

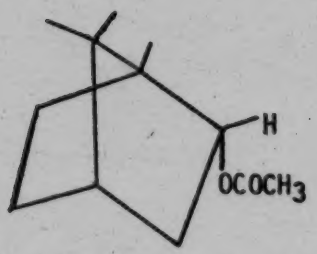


Fenchone

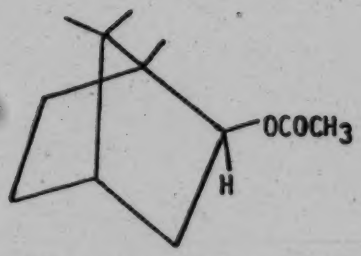
group in fenchone is located between a methyl and a gem-dimethyl group which causes a steric hindrance to the polarity of CO.

Among the acetate constituents, bornyl acetate was found to be more polar than isobornyl acetate. The difference between the two constituents is in the position of the $OCOCH_3$ group. Bornyl acetate

has an axial $OCOCH_3$ group whereas isobornyl acetate has an equatorial



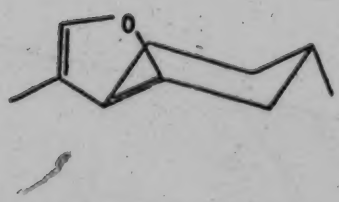
Bornyl acetate



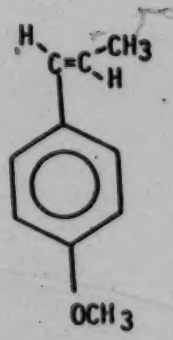
Isobornyl acetate

$OCOCH_3$ group. The steric hindrance of the methyl and gem-dimethyl groups on the polarity of $OCOCH_3$ group is greater in isobornyl acetate than in bornyl acetate.

Of the ether constituents, trans-anethole was found to be more polar than menthofuran. Trans-anethole is a benzene derivative with a



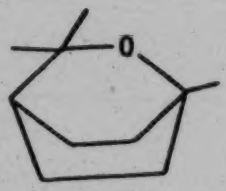
Menthofuran



trans-Anethole

methoxy group (OCH_3) and a double bond whereas menthofuran is an ether with two double bonds. Of interest is 1,8-cineol which is also an ether

but its spot was found among the constituents with OH groups. It seemed that 1,8-cineol behaved as an alcohol during the TLC development.



1,8-Cineol

However, there was no structural change of 1,8-cineol in TLC development as was proved by preparative TLC. Thus, there is a possibility of interaction between 1,8-cineol and silicic acid during the development, but without structural change of 1,8-cineol.

Among the hydrocarbon groups, p-cymene proved to be more polar than limonene because it has three double bonds whereas limonene has only two double bonds.

p-Cymene

Limonene

B. Thin-Layer Chromatography - Individual Oils

1. Anise Seed Oil

The thin-layer chromatogram of anise oil and some of its associated pure compounds is illustrated in Fig. 2. The shading indicates the intensity of the spots. Thirteen spots were obtained in the thin-layer chromatograms of anise oil from Brooks and Michigan. The difference between chromatograms of anise oil from these two origins was shown by the size and color intensity of these spots. Spots 13, 10, 9 and 6 of anise oil from Brooks were more intense in color and larger in size than those from Michigan. The opposite was true for spots 1, 4, 5 and 7. There were two major spots, numbers 12 and 13, for oil from Brooks but only one, number 12, for Michigan oil. The spots of anise oil were tentatively identified by running a chromatogram of anise oil and its associated standards and comparing the Rf values and colors. The Rf values and tentative identities of the spots of the chromatogram of anise oil are given in Table 7.

The results of the preparative thin-layer chromatography of anise oil from Brooks--1971 crop, are given in Table 8. The preparative TLC showed that the identities of spots 1, 2, 3 and 7 could not be confirmed because these constituents were either present in small amounts or absorbed too strongly by the silicic acid to be extracted by ethyl acetate. Spot 4 contained two minor constituents, peaks 16 and 17. The presence of anisyl acetone in spot 4 could not be confirmed because the GLC run was not long enough to detect it. Spots 5 and 6 were minor; the former contained borneol and the latter linalool. Spot 8 contained three constituents: carvone, p-anisaldehyde and a constituent which is not

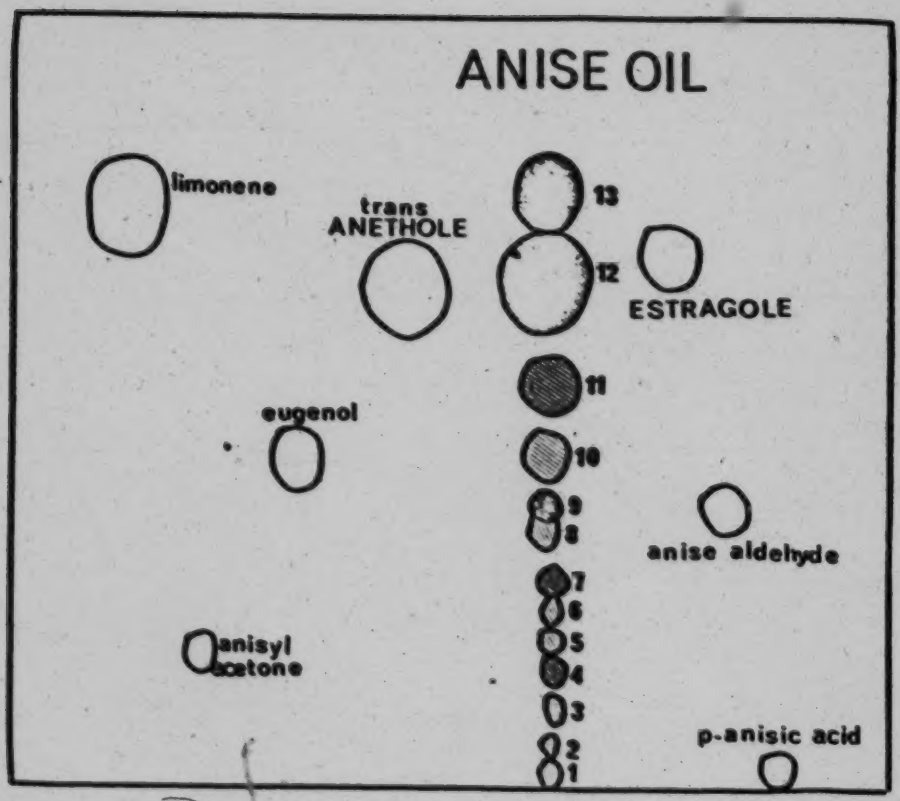


Fig. 2. Thin-Layer Chromatogram of Anise Seed Oil and Some of its Associated Pure Compounds.

Adsorbent layer: 300 μ of MN-Kieselgel N (Macherey Nagel & Co)
Solvent system: benzene-ethylacetate, 95:5 v/v.
Spot detection: spraying 1% vanillin in conc. sulfuric acid and heating in oven at 105° for 10 min.
Volume of pure oil applied: 1 μl.

TABLE 7

THIN-LAYER CHROMATOGRAPHY DATA OF ANISE SEED OILS

Spot Number	Rf Value*	Tentative Identity of the Spot
1	0.02	p-anisic acid
2	0.05	
3	0.12	
4	0.14	anisyl acetone
5	0.18	borneol
6	0.21	linalool
7	0.24	
8	0.32	carvone and p-anisaldehyde
9	0.35	fenchone
10	0.40	eugenol and camphor
11	0.47	thujone
12	0.61	trans-anethole and estragole
13	0.72	terpene hydrocarbons: limonene, myrcene, β-caryophyllene and p-cymene

*An average of at least ten separations

TABLE 8

PREPARATIVE THIN-LAYER CHROMATOGRAPHY DATA OF ANISE SEED OIL FROM BROOKS - 1971

Spot Number	Constituent*
1	
2	
3	
4	two minor constituents, 16 and 17
5	borneol
6	linalool
7	
8	carvone, p-anisaldehyde and a constituent in p-anisic acid
9	fenchone
10	camphor and eugenol
11	constituent 63, thujone, constituent 57 and 58 and p-cresol
12	trans-anethole, estragole, anisyl alcohol, constituents 54, 34(cis-anethole) and 50
13	terpene hydrocarbons: limonene, and β-caryophyllene, p-cymene and 11 other minor and 20 trace constituents

*The numbers refer to the peak numbers assigned on the gas-liquid chromatogram of the same oil sample

separated from p-anisic acid by GLC. Fenchone was present in spot 9. Spot 10 contained camphor and eugenol. There were five constituents in spot 11. The major constituent was peak 63. The other constituents were thujone, peaks 57 and 58, and p-cresol. Thujone was found more towards the upper part of the spot. Spot 12 contained trans-anethole, estragole, anisyl alcohol, constituents 54, 34 (cis-anethole) and 50, with trans-anethole and estragole being the major constituents of the spot. Constituent 34 (cis-anethole) was found more in the upper part of the spot with constituent 54 more in the lower part. Spot 13 contained the terpene hydrocarbons: limonene, and β-caryophyllene, p-cymene 11 minor and 20 trace constituents. Constituent 34 (cis-anethole) was in the lower part of the spot whereas constituents 26, 9 and 40 were more in the upper part of the spot. The spot for constituent 34 (cis-anethole) was located between spots 12 and 13.

2. Caraway Seed Oil

The thin-layer chromatogram of caraway seed oil and some of its pure compounds is illustrated in Fig 3. The chromatograms of caraway oil from Brooks and Michigan each showed 7 spots of which spots 4 and 7 were the major ones. The difference in the chromatograms of the two caraway oils was in the color intensity and the size of the spots. The color of spots 1, 2, 3 and 4 for Michigan oil was more intense and the size greater than the corresponding spots for Brooks oil. However, spot 7 was larger and more intense in color for the Brooks oil. The tentative identification of the spots was established in the usual manner by running a chromatogram of the oil and its associated standard components and comparing their Rf values and colors. The Rf values and tentative

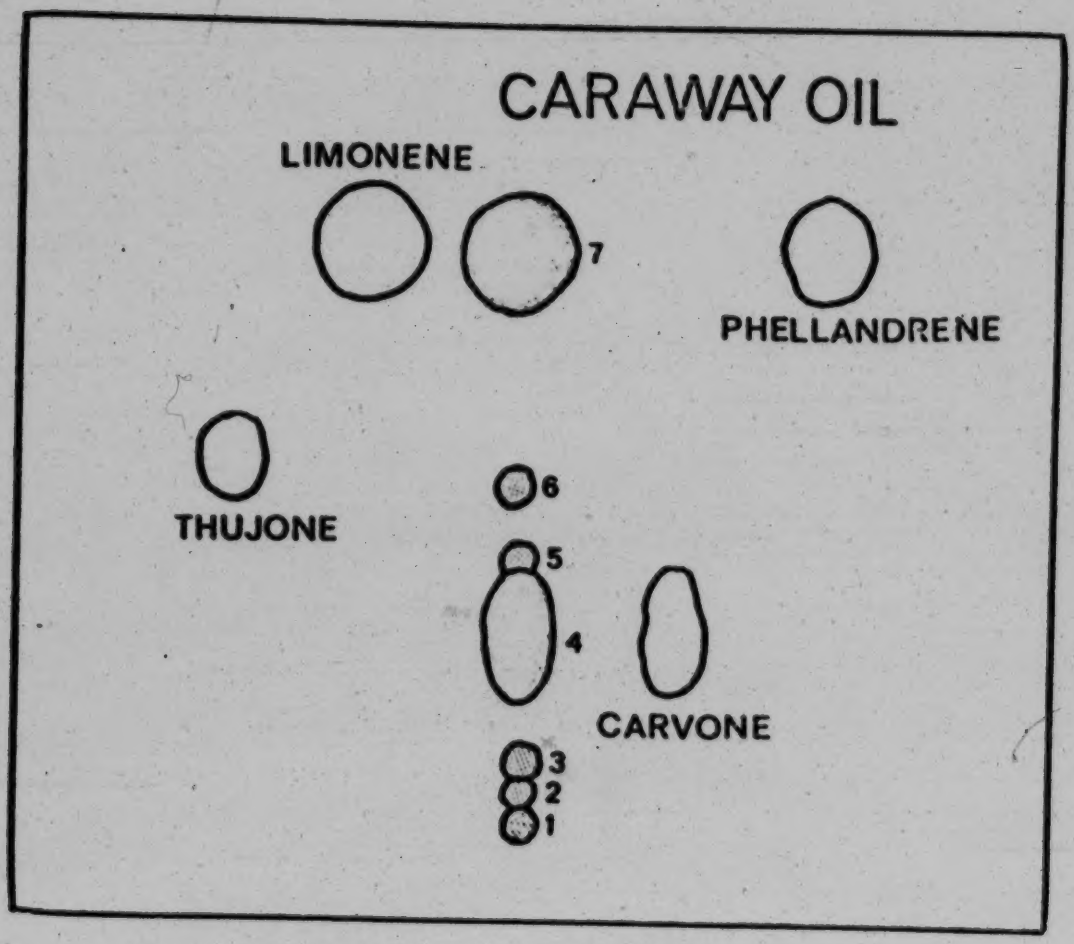


Fig. 3. Thin-Layer Chromatogram of Caraway Seed Oil and Some of its Associated Pure Compounds.

Adsorbent layer: 300 μ of MN-Kieselgel N (Macherey Nagel & Co)
Solvent system: benzene-ethylacetate, 95:5 v/v.
Spot detection: spraying 1% vanillin in conc. sulfuric acid and heating in oven at 105° for 10 min.
Volume of pure oil applied: 1 μl.

identities of the spots for caraway oil are given in Table 9.

The results of the preparative thin-layer chromatography of caraway oil from Brooks--1971 crop are shown in Table 10. The preparative TLC showed that spot 1 contained two minor constituents 34 and 41, while spot 2 contained five minor constituents, 35, 37, 38, 40 and 41. Thus, the difference in the identities of spot 1 and 2 was due to constituents 37, 38 and 40. Spot 3 contained only linalool. Spot 4 was

TABLE 9

THIN-LAYER CHROMATOGRAPHY DATA OF CARAWAY SEED OILS

Spot Number	Rf Value*	Tentative Identity
1	0.13	
2	0.16	
3	0.23	linalool
4	0.34	carvone
5	0.41	
6	0.49	thujone
7	0.70	terpene hydrocarbons: limonene, α-pinene, camphene, p-cymene, β-caryophyllene, etc.

*An average of at least ten separations

comprised of carvone, the major constituent, and three minor constituents, 37, 44 and 29. Constituent 44 was located towards the lower part of spot 4 whereas constituent 29 was found towards the upper part. There

TABLE 10

PREPARATIVE THIN-LAYER CHROMATOGRAPHY DATA OF CARAWAY SEED OIL FROM BROOKS - 1971 CROP

Spot Number	Constituents*
1	two minor constituents, 35 and 41
2	five minor constituents, 35, 37, 38, 40 and 41
3	linalool
4	carvone and three minor constituents, 29, 37 and 44
5	two minor constituents, 43 and 38
6	α- and β-thujone, fenchone, camphor and four other minor constituents, 43, 37, 32 and 25
7	terpene hydrocarbons: limonene, myrcene, α-pinene, β-pinene, α-phellandrene, β-caryophyllene and camphene and trace constituents, 30, 27, 11, 12, 14, 15, 16, 17, 47, 51 and 49 (trans-anethole, a minor constituent, was found between spots 6 and 7)

*The numbers refer to peak numbers assigned on the gas-liquid chromatogram of the same oil sample

were two minor constituents, 43 and 38, in spot 5. Spot 6 contained α- and β-thujone, fenchone, camphor and four minor constituents, 43, 37, 32 and 25. Spot 7 was comprised of terpene hydrocarbons which included limonene, myrcene, α-pinene, β-pinene, α-phellandrene, β-caryophyllene and camphene and minor constituents, 30, 27, 11, 12, 14, 15, 16, 17, 47, 51 and 49. Trans-anethole, a minor constituent, was found between spots 6 and 7.

3. Dill Oil

The thin-layer chromatogram of dill oil and some of its associated pure compounds is given in Fig. 4. The chromatograms of dill oils from Brooks and Michigan each showed a total of 8 spots. Dill prime and standard oils from Michigan and Danish dill oil from Brooks showed three major spots, numbers 9, 5 and 4, while the other two dill oils from Brooks showed only two major spots, numbers 9 and 5. The size of spot 4 was smaller for dill standard oil from Michigan and dill oil, 1972 crop, from Brooks. The size of spot 9 was bigger for both Michigan oils. The Rf values and the tentative identities of the spots of the thin-layer chromatogram of dill oil are given in Table 11.

The results of the preparative thin-layer chromatography of dill seed oil from Brooks 1971 crop are shown in Table 12. The preparative TLC of dill seed oil from Brooks did not bring about the identification of the constituents in spot 1. This was because the constituents either were present in traces or adsorbed too firmly to the silicic acid to be extracted by ethyl acetate. Spot 2 contained four minor constituents, 28, 30, 33 and 34, and spot 3 was comprised of two minor constituents, 38 and 23. Constituent 30, one of the major constituents of the dill oil, was present in spot 4. Carvone, the major constituent, and constituent 27 and two minor constituents, 36 and 42, were found in spot 5. Five minor constituents, 17, 18, 19, 24 and 15, were present in spot 6. Spot 7 contained one major constituent, 26, and four minor ones, 18, 24, 14 and 16. Trans-anethole and constituent 32 were found in spot 8. Constituent 32 was located in the lower part of the spot. Spot 9 contained the terpene hydrocarbons limonene, α-phellandrene, myrcene,

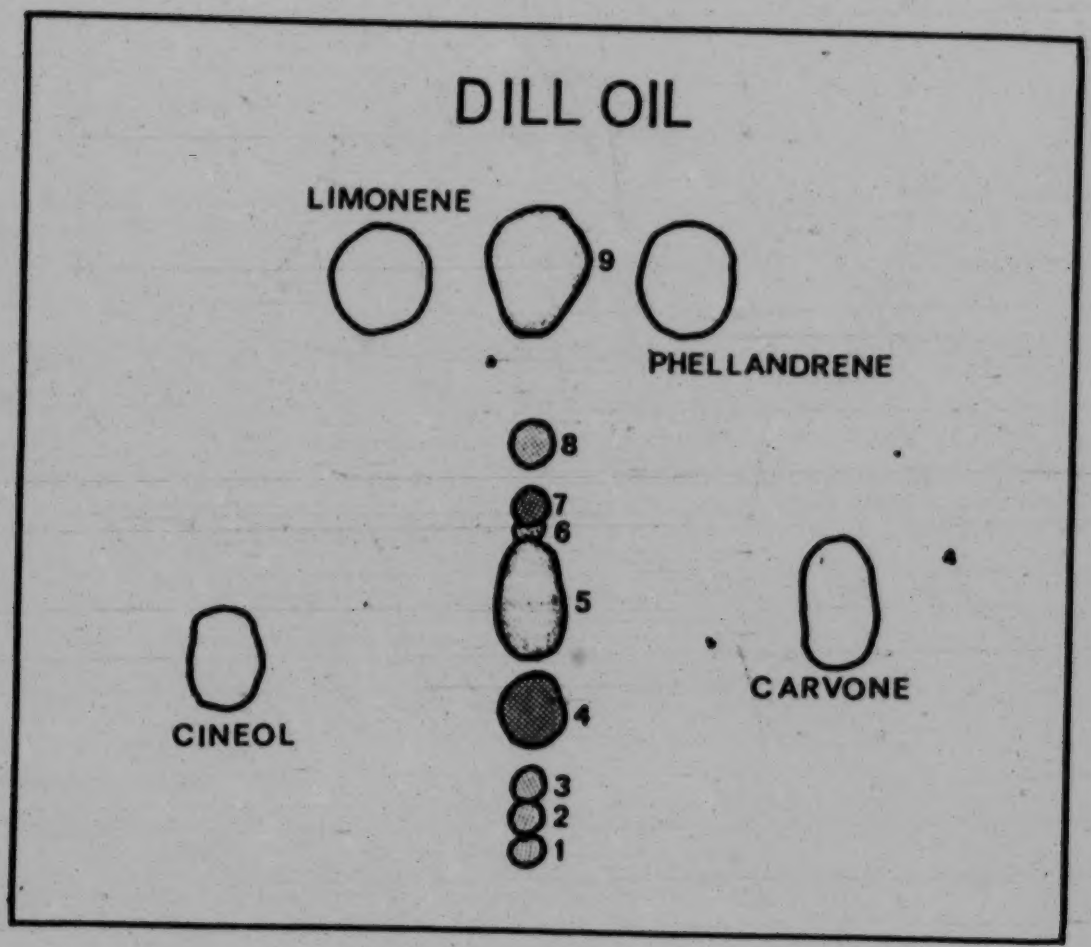


Fig. 4. Thin-Layer Chromatogram of Dill Oil and Some of its Associated Pure Compounds.

Adsorbent layer: 300 μ of MN-Kieselgel N (Macherey Nagel & Co)
Solvent system: benzene-ethylacetate, 95:5 v/v.
Spot detection: spraying 1% vanillin in conc. sulfuric acid and heating in oven at 105° for 10 min.
Volume of pure oil applied: 1 μl.

TABLE 11

THIN-LAYER CHROMATOGRAPHY DATA OF DILL OILS

Spot Number	Rf value*	Tentative Identity
1	0.11	
2	0.13	
3	0.17	
4	0.23	linalool and 1,8-cineol
5	0.35	carvone
6	0.43	
7	0.45	
8	0.50	thujone
9	0.70	terpene hydrocarbons: limonene, phellandrene, α-pinene, and myrcene and p-cymene

*An average of at least ten separations

TABLE 12

PREPARATIVE THIN LAYER CHROMATOGRAPHY DATA OF DILL OIL FROM BROOKS - 1971 CROP

Spot Number	Constituent*
1	
2	four minor constituents, 28, 30, 33 and 34
3	two minor constituents, 38 and 23
4	one major constituent, 20
5	carvone, constituent 27 and two minor constituents, 36 and 42
6	five minor constituents, 17, 18, 19, 24 and 15
7	one major constituent, 26, and four minor ones, 18, 24, 14 and 16
8	trans-anethole and constituent 32
9	terpene hydrocarbons: limonene, α-phellandrene, myrcene, α-pinene and β-pinene, p-cymene and constituents 4, 10, 11, 12, 22, 34, 15, 42, 40, 37 and 14

*The numbers refer to peak numbers assigned on a gas-liquid chromatogram for the same oil sample

α-pinene and β-pinene, and p-cymene and constituents 9, 10, 11, 12, 22, 34, 5, 42, 40, 37 and 14.

4. Fennel Seed Oil

The thin-layer chromatogram of fennel seed oil and some of its associated pure compounds is illustrated in Fig. 5. The thin-layer chromatograms of fennel oils from Brooks and Fritzsche, New York showed 10 and 11 spots, respectively, of which spots 10 and 11 were the major ones. Spot 1 was present only in the chromatogram of fennel oil from Fritzsche. The difference between the chromatograms of fennel oils from Brooks and Fritzsche also was in the size and intensity of the spots. The chromatogram of fennel oil from the Brooks 1971 crop showed that spots 11, 9 and 6 were larger and more intense in color, however, spot 10 was smaller than that of the Fritzsche and the Brooks 1972 crop. Spots 8, 2, 3 and 5 were larger and the color more intense for the fennel oil from Fritzsche than for Brooks' oils. Spots 8 and 7 became more distinct after charring the plate or leaving the plate at room temperature for a few days. The Rf values and tentative identities of the spots of the thin-layer chromatogram of fennel oil are shown in Table 13.

The results of the preparative thin-layer chromatography of fennel oil from Brooks 1971 crop are given in Table 14. The constituents in spot 3 could not be identified as they either were present in traces or adsorbed too firmly to the silicic acid. There was no spot 1 in the chromatogram of fennel oil from Brooks. A minor constituent, 32, was present in spot 2. Spot 4 contained a minor constituent which was not separated from carvone peak by gas-liquid chromatography. There were

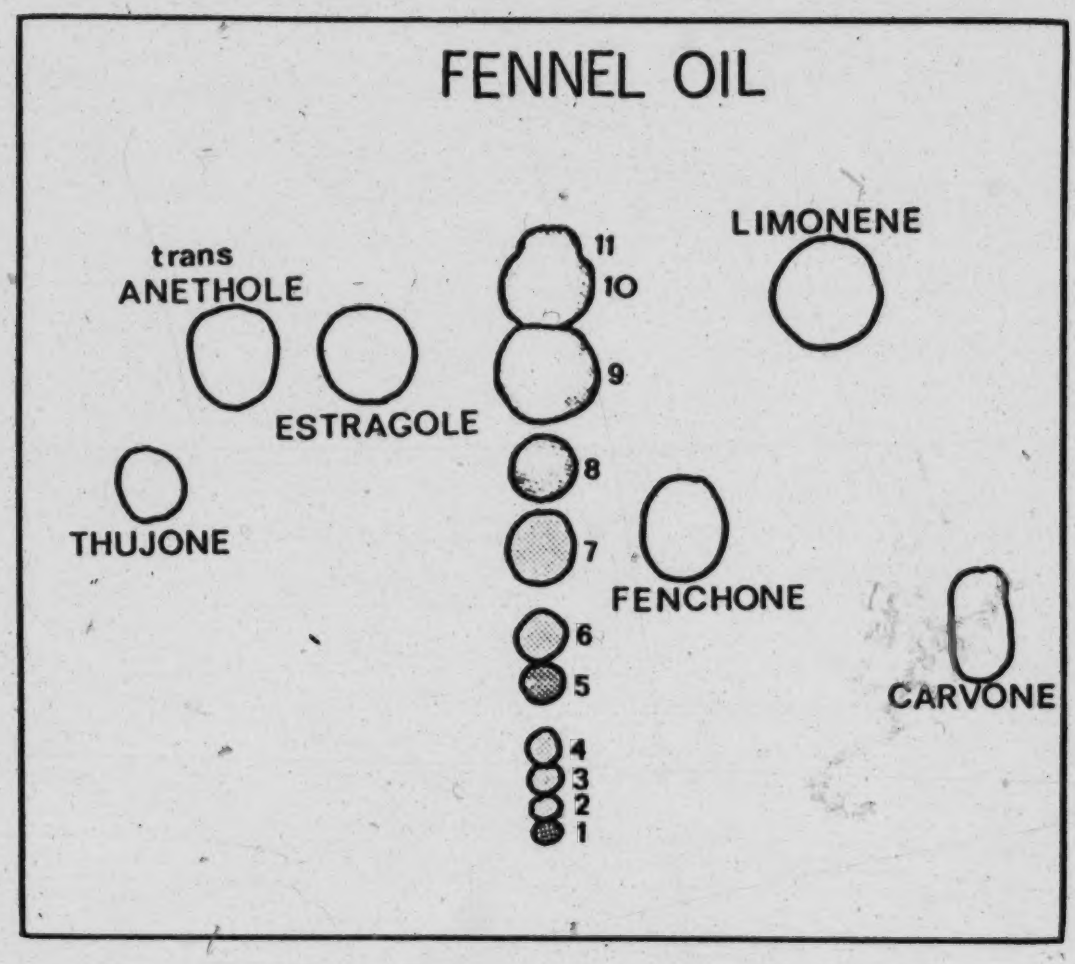


Fig. 5. Thin-Layer Chromatogram of Fennel Seed Oil and Some of its Associated Pure Compounds.

Adsorbent layer: 300 μ of MN-Kieselgel N (Macherey Nagel & Co)
Solvent system: benzene-ethylacetate, 95:5 v/v.
Spot detection: spraying 1% vanillin in conc. sulfuric acid and heating in oven at 105° for 10 min.
Volume of pure oil applied: 1 μl.

TABLE 13

THIN LAYER CHROMATOGRAPHY DATA OF FENNEL SEED OILS

Spot Number	Rf value*	Tentative Identity
1	0.05	p-anisic acid
2	0.11	
3	0.13	
4	0.17	
5	0.20	
6	0.25	carvone
7	0.32	fenchone
8	0.40	camphor
9	0.48	thujone
10	0.59	trans-anethole and estragole
11	0.70	terpene hydrocarbons: limonene, myrcene, α-pinene, α-phellandrene, etc.

*An average of at least ten separations

TABLE 14

PREPARATIVE THIN-LAYER CHROMATOGRAPHY DATA OF FENNEL SEED OIL FROM BROOKS - 1971 CROP

Spot Number	Constituent*
1	
2	a minor constituent, 32
3	
4	a minor constituent which was not separated from carvone peak by GLC
5	two minor constituents, 1,8-cineol and 17
6	carvone and two minor constituents, 34 and anisaldehyde
7	fenchone and three minor constituents, 28, 34 and 41
8	camphor and two minor constituents, 30 and 36
9	thujone and four minor constituents, 40, 34, 47 and 48
10	trans-anethole, estragole and three other constituents, 45,(cis-anethole), 60 and 65
11	terpene hydrocarbons: limonene, myrcene, α-phellandrene and α-pinene, p-cymene and minor constituents 17, 16, 42 (not estragole), 43, 50, 20, 48, 47, 58, 35 and 37

*The numbers correspond to peak numbers assigned on a gas-liquid chromatogram for the same oil sample

two minor constituents, 1,8-cineol and peak 17, present in spot 5. Carvone was the major constituent present in spot 6. The other two minor constituents present were anisaldehyde and peak 34. Spot 7 was comprised of fenchone and three minor constituents, 28, 34 and 41. Camphor and two minor constituents, 30 and 36, were present in spot 8. Spot 9 contained thujone as a major component, and four minor constituents, 40, 34, 47 and 48. Trans-anethole, estragole and three other constituents, 45 (cis-anethole), 60 and 65 were found in spot 10. Of the five constituents in spot 10, trans-anethole was the major one. Spot 11 was comprised of terpene hydrocarbons: limonene, myrcene, α-phellandrene and α-pinene, p-cymene and other minor constituents, 17, 16, 42 (not estragole), 43, 56, 20, 48, 47, 58, 35 and 37.

5. Peppermint Oil

The thin-layer chromatogram of peppermint oil and some of its associated pure compounds is illustrated in Fig. 6. The chromatograms of the oils from Brooks and Hotchkiss showed 11 spots of which spots 11, 9, 8 and 2 were the major ones. There was no distinct difference in the size and color intensity of the spots found on the chromatograms of the two oils. Spots 7 and 8 became more distinct after charring the sprayed plate or leaving the plate at room temperature for a few days. The Rf values and tentative identities of the spots for peppermint oil are shown in Table 15.

The results of the preparative thin-layer chromatography of Michigan peppermint oil from Hotchkiss are given in Table 16. The preparative TLC of this peppermint oil showed that spot 1 contained three minor constituents, 24, 50 and 51. Menthol was the major constituent in spot 2 which in addition contained 3-octanol, isomenthol and two

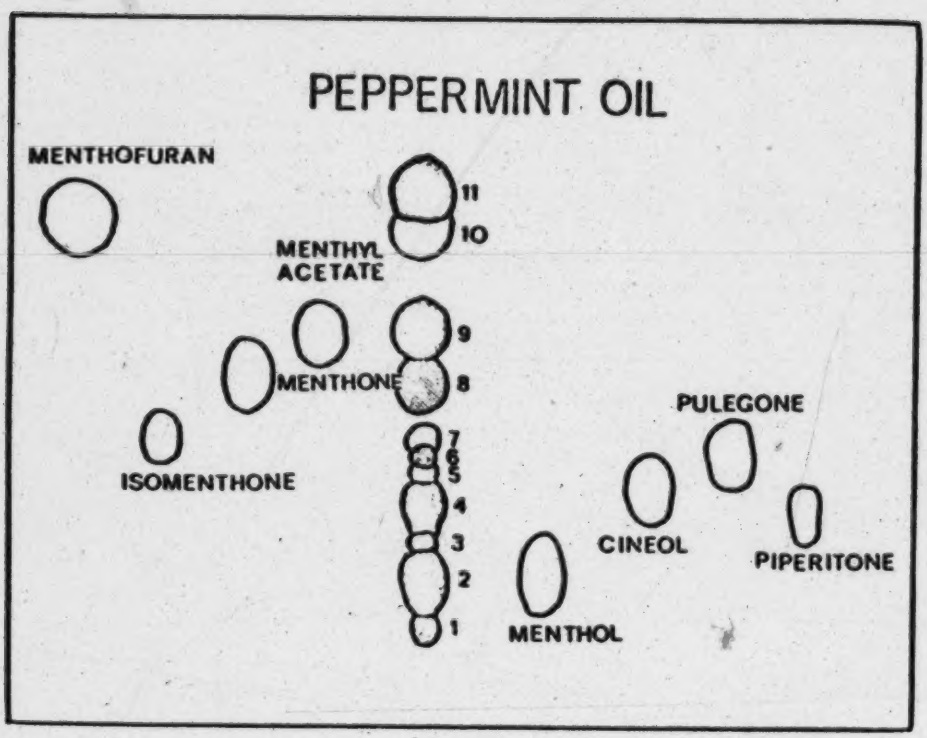


Fig. 6. Thin-Layer Chromatogram of Peppermint Oil and Some of its Associated Pure Compounds.

Adsorbent layer: 300 μ of MN-Kieselgel N (Macherey Nagel & Co)
Solvent system: benzene-ethylacetate, 95:5 v/v.
Spot detection: spraying 1% vanillin in conc. sulfuric acid and heating in oven at 105° for 10 min.
Volume of pure oil applied: 1 μl.

TABLE 15

THIN LAYER CHROMATOGRAPHY DATA OF PEPPERMINT OILS

Spot Number	Rf Values*	Tentative Identity
1	0.13	
2	0.20	menthol
3	0.26	piperitone
4	0.29	neomenthol
5	0.33	1,8-cineol
6	0.35	pulegone
7	0.38	isomenthone
8	0.46	menthone
9	0.53	menthyl acetate
10	0.67	menthofuran
11	0.72	terpene hydrocarbons: limonene, myrcene, etc.

*An average of at least ten separations

minor constituents, 35 and 25. Piperitone was found in spot 3. The constituents detected in spot 4 were neomenthol, 1,8-cineol, neoisomenthol and linalool. 1,8-Cineol and neoisomenthol were present in spot 5. Spot 6 contained pulegone, and spot 7 isomenthone. Spot 8 was comprised of menthone and constituent 41 which was found in the upper part of the spot. Menthyl acetate, the major constituent, and two minor constituents,

TABLE 16

PREPARATIVE THIN LAYER CHROMATOGRAPHY DATA OF MICHIGAN PEPPERMINT OIL FROM HOTCHKISS

Spot Number	Constituent*
1	three minor constituents, 24, 50 and 51
2	menthol, 3-octanol, isomenthol and two minor constituents, 35 and 25
3	piperitone
4	neomenthol, 1,8-cineol, neoisomenthol and linalool
5	1,8-cineol and neoisomenthol
6	pulegone
7	isomenthone
8	menthone and constituent 41
9	menthyl acetate and two other minor constituents, 23 and 35
10	menthofuran and one other constituent
11	terpene hydrocarbons: β-caryophyllene, myrcene, limonene, α-pinene, β-pinene and camphene, p-cymene and seven minor constituents, 48, 46, 45, 32, 13, 14 and 16

*The numbers correspond to peak numbers assigned on gas-liquid chromatogram for the same oil sample

23 and 35, were found in spot 9. Spot 10 contained menthofuran and a constituent which was not separated from peak 11 by GLC. The terpene hydrocarbons β-caryophyllene, myrcene, limonene, α-pinene, β-pinene and camphene were present in spot 11 along with p-cymene and seven other constituents, 48, 46, 45, 32, 13, 14 and 16.

The thin-layer chromatogram of menthone related constituents together with their Rf values and configurations is illustrated in Fig. 7. With the solvent system used the Rf values were: for menthol 0.20, neomenthol 0.29, isomenthone 0.38, menthone 0.46, menthyl acetate 0.53, and menthofuran 0.67. The Rf values of isomenthol, neoisomenthol and neomenthyl acetate could not be exactly determined but their positions on the chromatogram were found by preparative TLC. Menthol was not well separated from isomenthol, nor was neomenthol from neoisomenthol or menthyl acetate from neomenthyl acetate. However, preparative TLC showed that isomenthol migrated slower than menthol, and neomenthyl acetate slower than menthyl acetaee, while neoisomenthol travelled faster than neomenthol. Petrowitz (1960) used four solvent systems to study the separation of menthol stereoisomers and obtained the results given in Table 17.

The separation of the menthone-related constituents depends on polarity differences due to variation of functional groups, stereochemistry and solvent system. Petrowitz (1960) found that the molecules with equatorial hydroxyl groups, as in menthol and isomenthol, showed a higher adsorption affinity with benzene. However, the effect of benzene on methyl groups, whether in equatorial or axial positions, was less significant. With chloroform as the solvent system neoisomenthol migrated

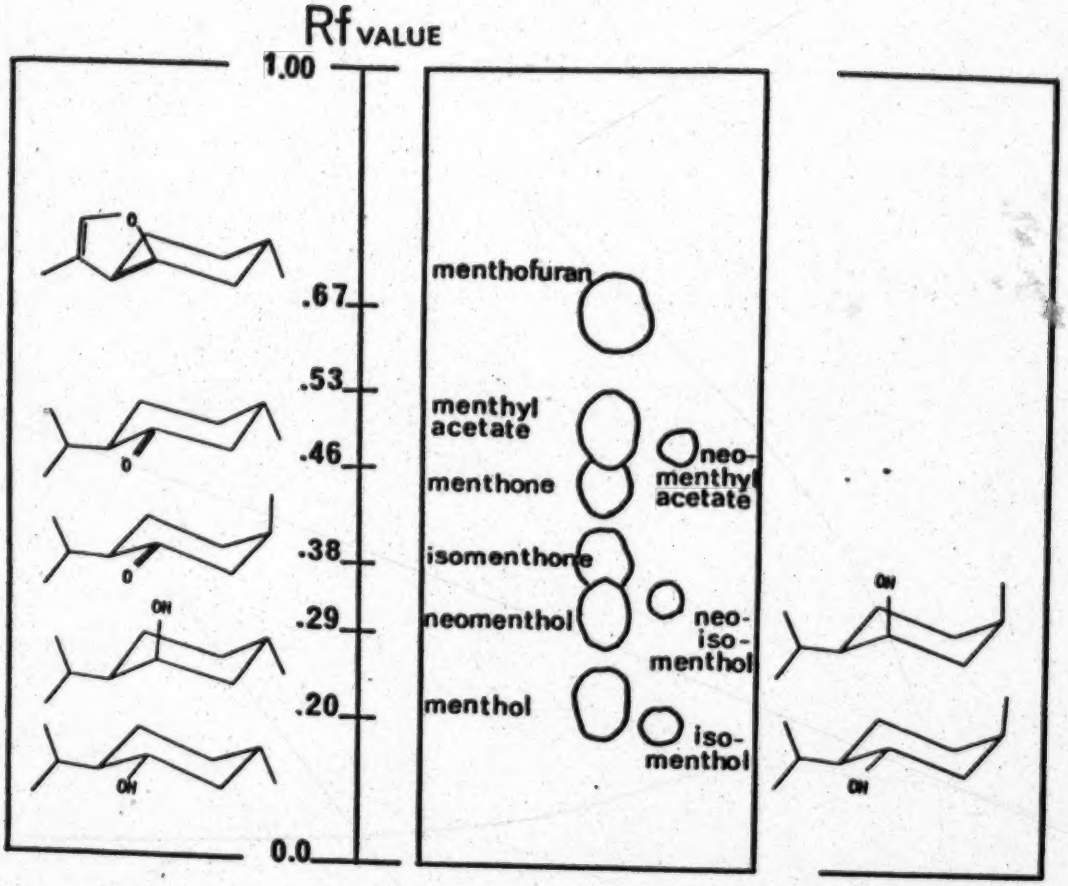


Fig. 7. Thin-Layer Chromatogram of Menthone Related Constituents.

Adsorbent layer: 300 μ of MN-Kieselgel N (Macherey Nagel & Co)
Solvent system: benzene-ethylacetate, 95:5 v/v.
Spot detection: spraying 1% vanillin in conc. sulfuric acid and heating in oven at 105° for 10 min.
Volume of pure oil applied: 1 μl.

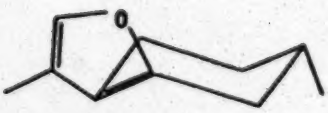
TABLE 17

THIN LAYER CHROMATOGRAPHY DATA OF THE MENTHOL STEREOISOMERS

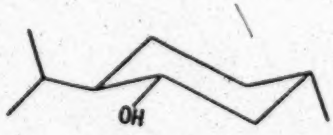
Compound	Rf Value			
	Chloroform	n-hexane:ethyl acetate (90:10)	Benzene: methanol (75:25)	Benzene: methanol (95:5)
Menthol	0.32	0.47	0.67	0.36
Isomenthol	0.29	0.41	0.62	0.37
Neomenthol	0.40	0.64	0.73	0.51
Neoisomenthol	0.36	0.68	0.76	0.55

slower than neomenthol, but the opposite was true with the other three solvent systems.

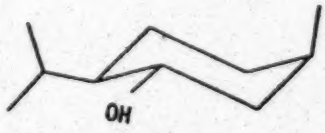
The functional groups in menthone-related constituents are: OH, CO, $OCOCH_3$ and COC, in decreasing order of polarity. Hence, menthols are the most polar and menthofuran is the least polar. The difference between isomenthol and menthol is the position of the methyl group, which is equatorial in menthol and axial in isomenthol. With the methyl



Menthofuran

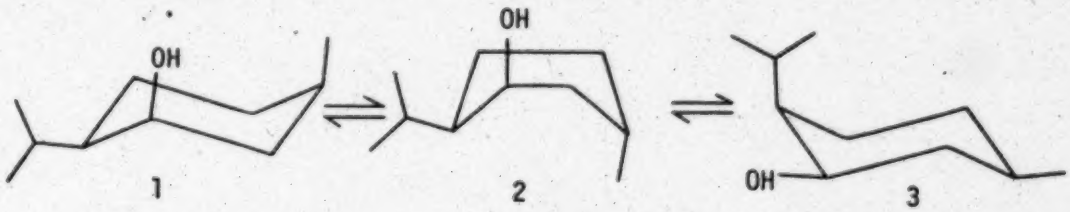


Menthol



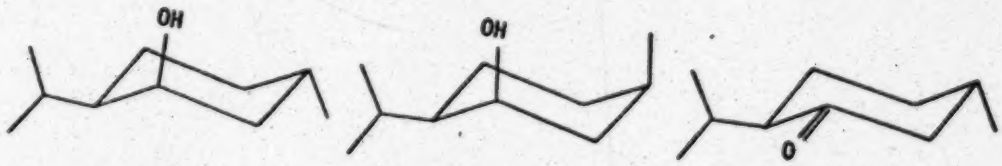
Isomenthol

group in the equatorial position there is greater steric hindrance with the OH group and, hence, the polarity of menthol is slightly reduced when compared with that of isomenthol. However, for neomenthol and neoisomenthol which appear to be less polar than menthol and isomenthol, their polarities might be disputed, since these compounds might be present in conformational equilibrium as illustrated for neoisomenthol.



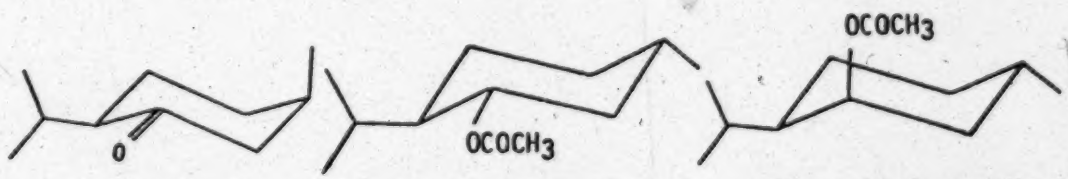
Neoisomenthol

The conformation 1 is less polar than conformation 3, hence the overall separation characteristic for neoisomenthol will depend on the position of equilibrium, which should be in favor of a more stable conformation.



Neomenthol Neoisomenthol Menthone

Isomenthone is more polar than menthone because its methyl group is in the axial position, whereas the



Isomenthone Menthyl acetate Neomenthyl acetate

methyl group in menthone is in the equatorial position. Of the two acetates, menthyl acetate is more polar than neomenthyl acetate because the former has its $OCOCH_3$ in the equatorial position while the latter has its $OCOCH_3$ in the axial position.

6. Sage Oil

A thin-layer chromatogram of sage oil and some of its associated pure compounds is illustrated in Fig. 8. The chromatograms of sage oil from Brooks and Michigan each showed 9 spots of which seven spots were major. The minor spots were spots 1 and 2. Spots 3 and 5 of the Brooks' oil were less intense in color and smaller in size relative to the Michigan oil, but the opposite was true for spots 4, 6 and 9. Spots 6 and 7 became more distinct after charring the plate or leaving the plate at room temperature for a few days. The Rf values and tentative identities of the spots of the thin-layer chromatogram of sage oil are shown in Table 18.

The results of the preparative thin-layer chromatography of sage oil from the Brooks 1971 crop are given in Table 19. From the preparative chromatogram, the constituents present in spot 1 could not be identified. Spot 2 contained borneol and two minor constituents, while spot 3 contained borneol and eleven minor constituents. These two spots could be differentiated by their minor constituents. The constituents in spot 4 were linalool and three other constituents, one of which decomposed at about 150°. Spot 6 contained camphor and eight minor constituents, one of which decomposed at about 185°. Spot 7 was comprised of α- and β-thujone and three minor constituents. Bornyl acetate and three minor constituents were found in spot 8. The constituents in spot 9 were the

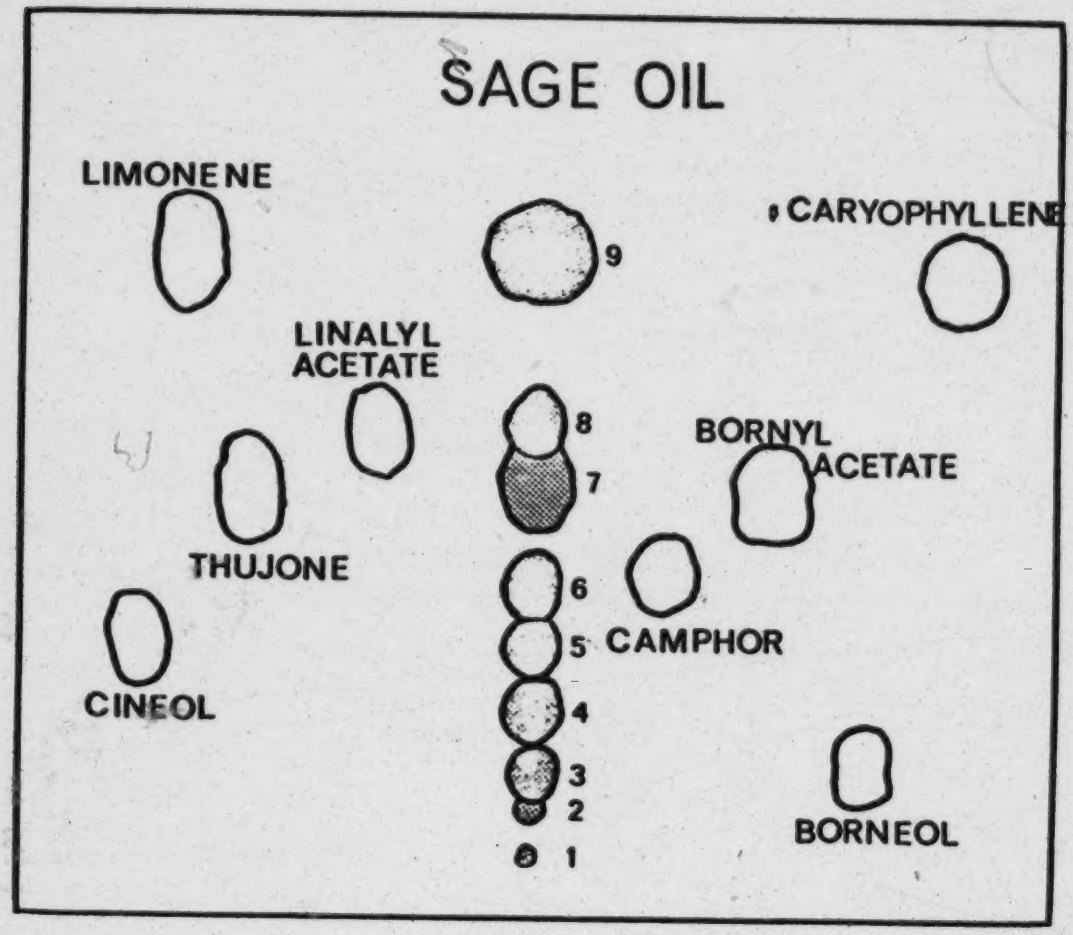


Fig. 8. Thin-Layer Chromatogram of Sage Oil and Some of its Associated Compounds.

Adsorbent layer: 300 µ of MN-Kieselgel N (Macherey Nagel & Co)
Solvent system: benzene-ethylacetate, 95:5 v/v.
Spot detection: spraying 1% vanillin in conc. sulfuric acid and heating in oven at 105° for 10 min.
Volume of pure oil applied: 1 µl.

terpene hydrocarbons: humulene, limonene, myrcene, α-pinene, β-pinene, α-thujene and camphene, as well as p-cymene and thirteen minor constituents.

TABLE 18

THIN-LAYER CHROMATOGRAPHY DATA OF SAGE OILS

Spot Number	Rf Value*	Tentative Identity
1	0.07	
2	0.12	
3	0.15	L-borneol
4	0.21	linalool
5	0.27	1,8-cineol
6	0.34	camphor
7	0.44	thujone
8	0.51	bornyl acetate
9	0.70	terpene hydrocarbons: limonene, humulene, camphene, myrcene, phellandrene, etc.

*An average of at least ten separations

TABLE 19

PREPARATIVE THIN LAYER CHROMATOGRAPHY DATA OF
SAGE OIL FROM BROOKS - 1971 CROP

Spot Number	Constituent
1	
2	borneol and two minor constituents
3	borneol and eleven minor constituents
4	linalool and three other constituents
5	1,8-cineol
6	camphor and eight minor constituents
7	α- and β-thujone and three minor constituents
8	bornyl acetate and three minor constituents
9	terpene hydrocarbons: humulene, limonene, myrcene, α-pinene, β-pinene, camphene and α-thujene, and p-cymene and thirteen minor constituents

C. Infrared

1. Anise Seed Oil

The infrared spectrum of anise seed oil from the Brooks 1971 crop is illustrated in Fig. 9. The spectra of anise seed oil from Brooks 1972 crop and Michigan are given in Appendix B-1. The spectrum of anise oil was dominated by the spectra of trans-anethole, the major constituent, and other benzene derivative constituents. The bands in this spectrum could be associated with those typical of trans-anethole, cis-anethole, eugenol, p-anisaldehyde, limonene, p-cymene, thymol, anisyl alcohol, estragole, camphor, linalool and safrole.

2. Caraway Seed Oil

The infrared spectrum of caraway seed oil from Brooks--1971 crop is illustrated in Fig. 10. The spectrum of caraway oil from Michigan is given in Appendix B-2. The spectra of two major components in caraway oil, carvone and limonene, dominated the spectrum of the oil. The bands in this spectrum could be associated with those bands typical of limonene, carvone, p-cymene, 1,8-cineol, α-pinene, β-pinene, β-phellandrene, myrcene, trans-anethole, camphor, estragole, fenchone and acetates.

3. Dill Oil

The infrared spectrum of Danish dill seed oil from Brooks--1971 crop is illustrated in Fig. 11. The spectra of common dill seed oil from Brooks--1971 crop and dill standard and prime from Michigan are

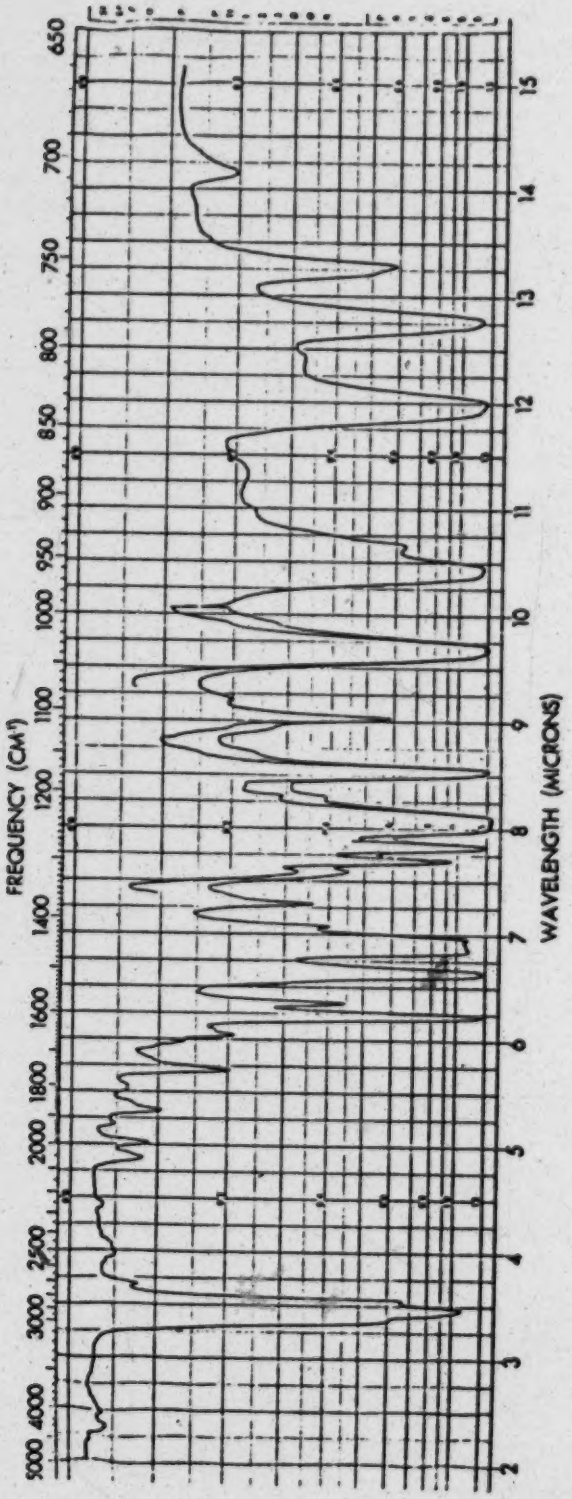


Fig. 9. Infrared Spectrum of Anise Seed 011 (Brooks--1971 Crop).

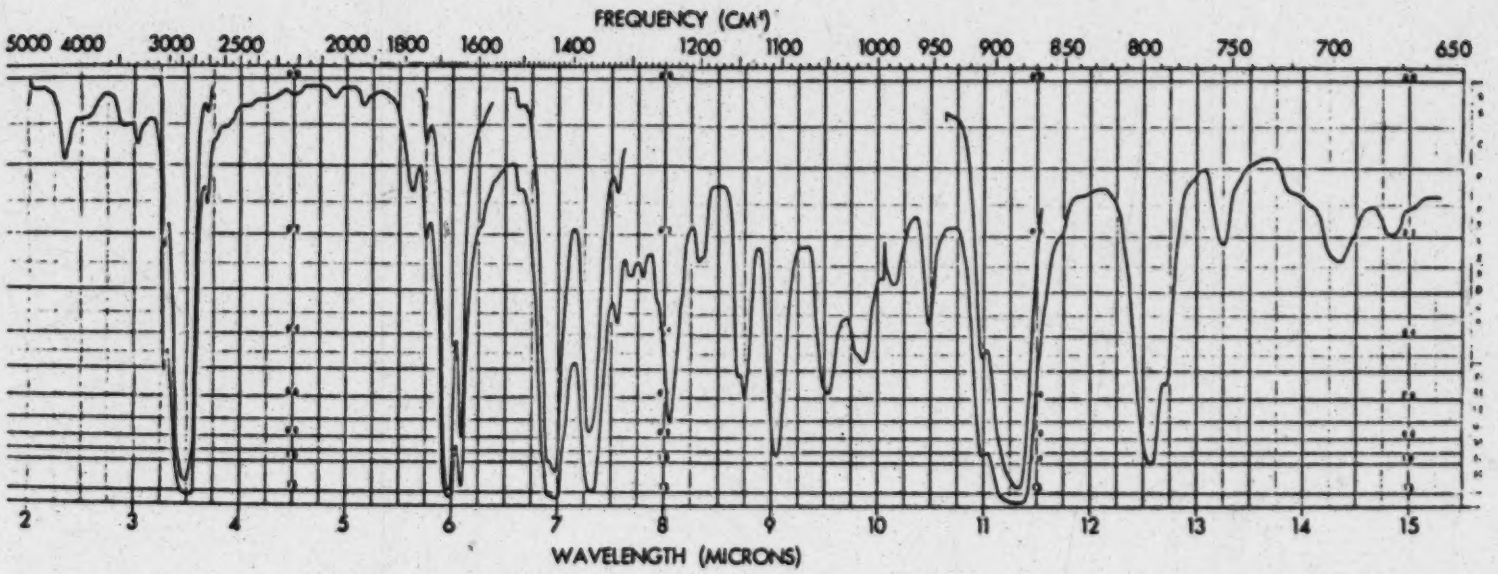


Fig. 10. Infrared Spectrum of Caraway Seed Oil (Brooks--1971 Crop).

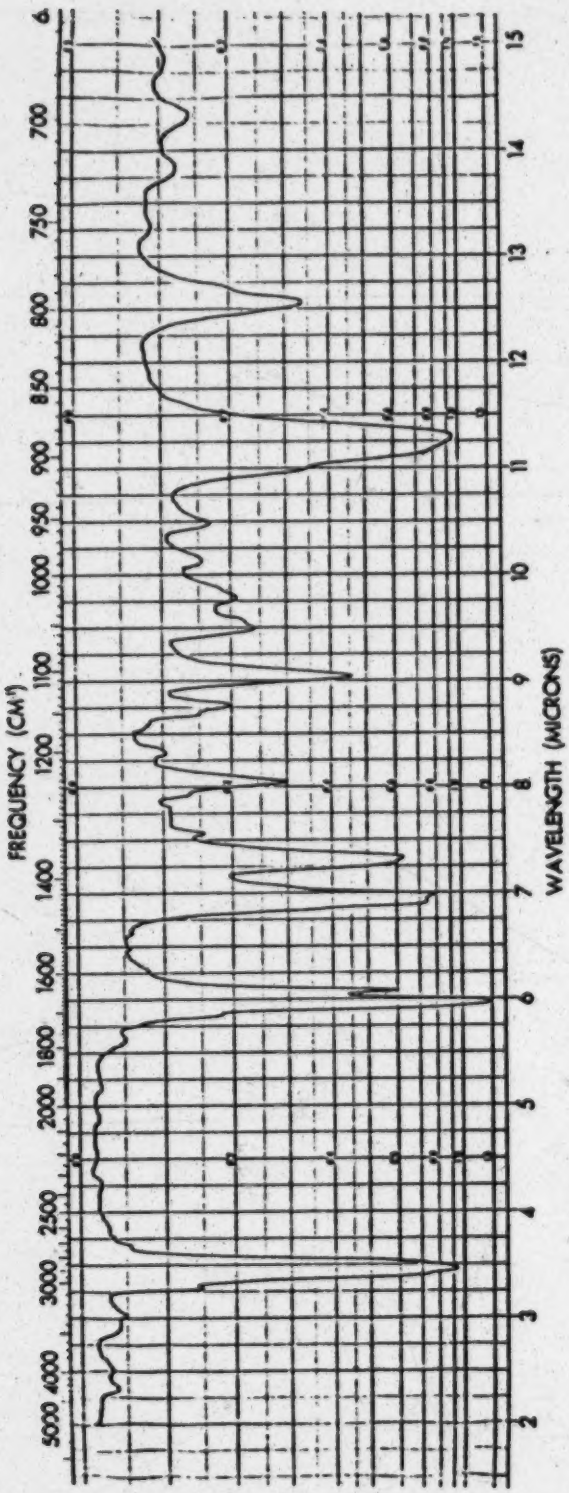


Fig. 11. Infrared Spectrum of Danish D111 Seed Oil (Brooks--1971 Crop).

given in Appendix B-3. The spectra of two major constituents in dill oil, limonene and carvone, dominated the spectrum of the oil. The bands in this spectrum could be associated with those typical of limonene, carvone, p-cymene, 1,8-cineol, α-pinene, β-phellandrene, trans-anethole, camphor, myrcene, fenchone, γ-terpinene and acetates.

4. Fennel Seed Oil

The infrared spectrum of fennel seed oil from Brooks--1971 crop is shown in Fig. 12. The spectra of fennel oils from Brooks--1972 crop and Fritzsche ane given in Appendix B-4. The spectrum of fennel oil was dominated by the spectra of its major constituents, trans-anethole, limonene and fenchone. The bands in this spectrum could be associated with those typical of trans-anethole, limonene, fenchone, camphor, p-cymene, p-anisaldehyde, α-pinene, thujone, estragole, camphene, β-pinene, linalool, β-phellandrene and fenchyl alcohol.

5. Peppermint Oil

The infrared spectrum of leaf peppermint oil, stage I, from Brooks--1970 crop is shown in Fig. 13. The spectra of the leaf peppermint oils for stages II, III, IV and V, and the stem peppermint oils for stages I, III and Vffrom Brooks and peppermint oil from Michigan are given in Appendix B-5. The spectra of two major constituents of peppermint oil, menthol and menthone, dominated the

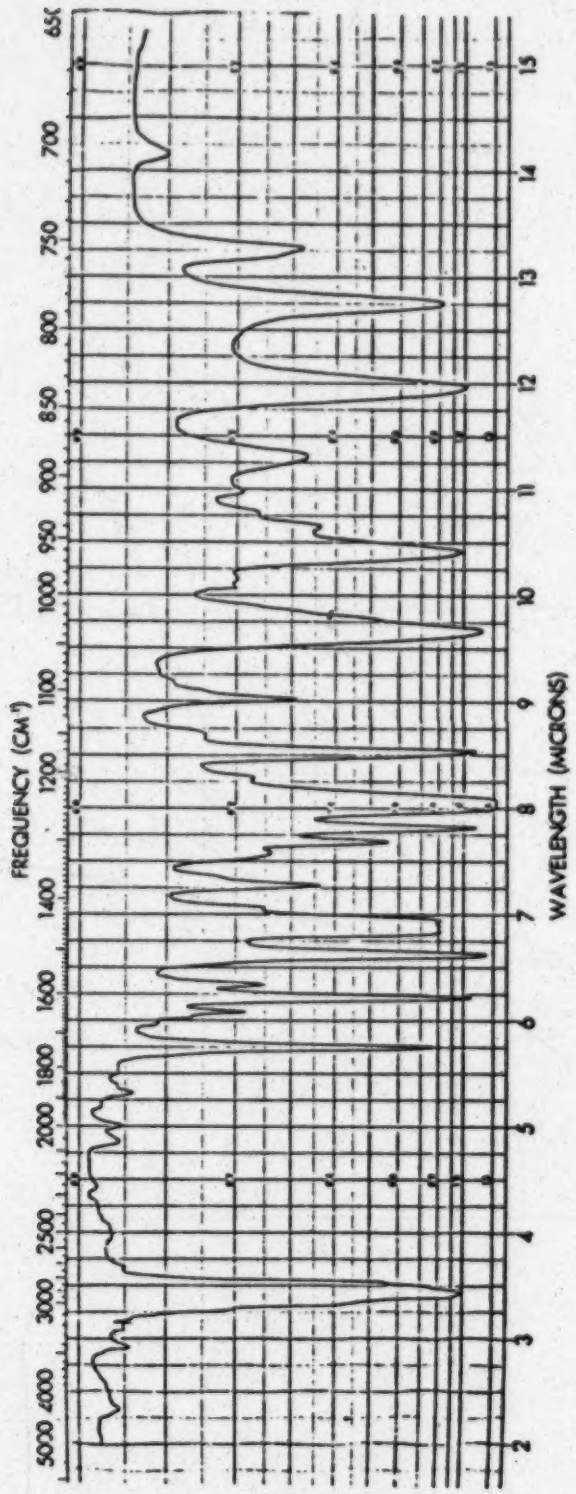


Fig. 12. Infrared Spectrum of Fennel Seed 011 (Brooks--1971 Crop).

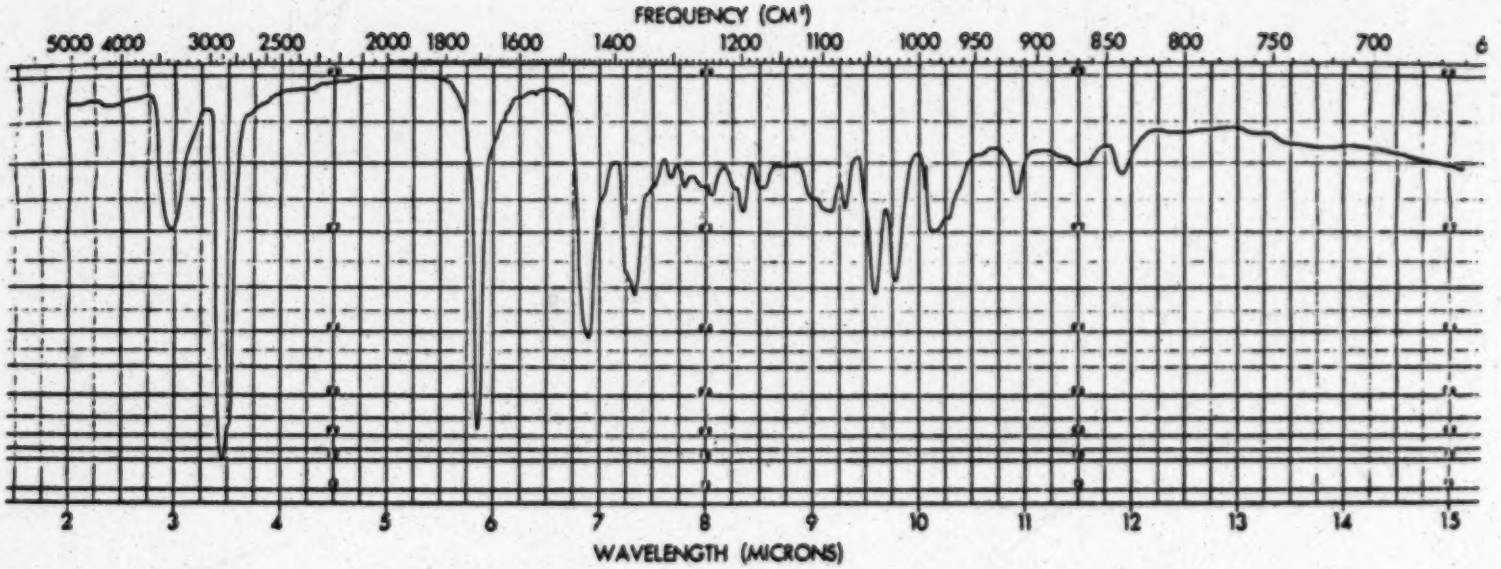


Fig. 13. Infrared Spectrum of Peppermint Oil
(Leaf, Stage I, Brooks--1970 Crop)

spectrum of the oil. The bands in this spectrum could be associated with those typical of menthol, menthone, pulegone, menthyl acetate, camphene, menthofuran, β-pinene, β-phellandrene, myrcene, p-cymene, camphor, 1,8-cineol, piperitol, limonene, isopulegol, and α-pinene.

6. Sage Oil

The infrared spectrum of sage oil from Brooks--1971 crop is illustrated in Fig. 14. The spectrum of sage oil from Michigan is given in Appendix B-6. The spectrum of sage oil was dominated by the spectra of its major constituents, camphor, thujone, 1,8-cineol and caryophyllene. The bands in this spectrum could be associated with those typical of camphor, thujone, 1,8-cineol, linalool, linalyl acetate, α-terpineol, bornyl acetate, camphene, β-pinene, β-caryophyllene, borneol, γ-terpinene, p-cymene, α-pinene, β-phellandrene and geranyl acetate.

D. Gas-Liquid Chromatography

1. The Separation of Individual Oil Constituents

a. General Observations

The separation of compounds by a GLC column depends on interactions occurring between substances in the gas, liquid and stationary phases. These interactions are directly dependent on the nature of these substances (Haken, 1973). Wehrli and Kovats (1959) suggested that in a nonpolar liquid phase the molecules are only affected by dispersion forces, but in a polar liquid phase there are two additional factors, namely, forces due to polar groups and to chemical bonds (hydrogen bond

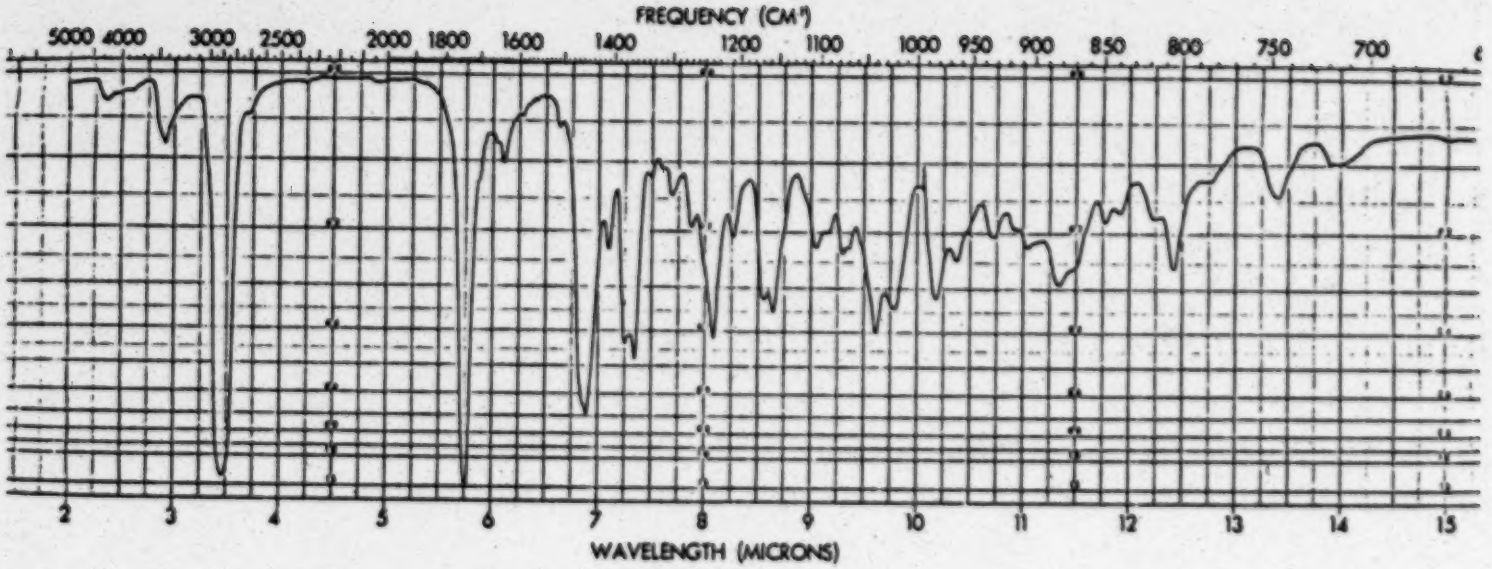


Fig. 14. Infrared Spectrum of Sage 011 (Brooks--1971 Crop).

and complex formation). Thus, the elution sequence of compounds during a GLC separation depends on the polarity of the liquid phase. The nonpolar liquid phases such as didecyl phthalate (Zubyk and Conner, 1960), Apiezon L and silicone oil (Klouwen and Heide, 1962) are nonselective. The elution order of essential oil constituents on these nonpolar liquid phases follows the boiling points of these components. However, the elution order on Carbowax 4000, a polar liquid phase, is different because Carbowax 4000 shows some selective retention for unsaturated compounds with conjugated double bonds (Zubyk and Conner, 1960). The elution sequence of the essential oil constituents on nonpolar (Silicone D.C. 550) and polar (Carbowax 20M) liquid phases is given in Table 26. In this study, ethylene glycol succinate (EGS), a polar liquid phase, was used. It was shown by Luisetti and Yunes (1971) that EGS is more polar than Carbowax 20M.

The elution sequence of a functional group class depends on the nature of the polar liquid phase. In general, the elution sequence on the EGS column was COC, CO, $OCOCH_3$ and OH. Bierl et al. (1972) reported that with diethylene glycol succinate (DEGS), the order of elution was COC, C-C, $OCOCH_3$, CO and OH, while the elution sequence on cyanopropylmenthyl phenyl-methyl silicone (OV-225) was COC, $OCOCH_3$, C-C, OH and CO.

In an earlier report Martin (1961, 1963) observed that the elution order of the constituents changed with the amount of liquid phase. In our study, it was found that the elution sequence differed on the fresh and on the aged columns. The amount of liquid phase in the aged column was expected to be less than that of the fresh column because of column bleeding. The change in the elution order of the constituents

TABLE 20

THE ELUTION SEQUENCE OF ESSENTIAL OIL CONSTITUENTS ON NONPOLAR AND POLAR LIQUID PHASES

Nonpolar Liquid Phase Silicone D.D. 550*		Polar Liquid Phase Carbowax 20M**	
Elution Sequence	Boiling Point (°C)	Elution Sequence	Boiling Point (°C)
α-pinene	157	α-thujene	152
camphene	158-9	α-pinene	157
β-pinene	164-6	camphene	158-9
myrcene	167	β-pinene	164-6
Δ3-carene (d)	172	sabinene	163-5
limonene	177	myrcene	167
γ-terpinene	183	α-terpinene	176
p-cymene	177-9	limonene	177
linalool	198	1,8-cineol	175
α-terpineol	219	β-phellandrene	171-8
linalyl acetate	220	cis-ocimene	177
D-carvone	230	p-cymene	177-9
		terpineolene	184
		menthone	204
		menthofuran	196
		isomenthone	208
		menthyl acetate	227
		neomenthol	212
		neoisomenthyl acetate	
		menthol	216
		pulegone	224
		piperitone	233

Data have been compiled from separations done by:
*Cartoni and Liberti (1960)
**Lawrence et al. (1972)

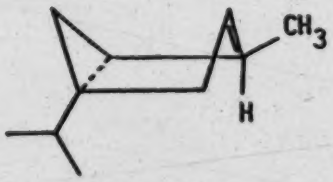
happened only among the oxygenated monoterpenes and sesquiterpenes. For instance, β-caryophyllene was eluted before menthol when peppermint oil was chromatographed on the fresh column, however, it appeared after menthol when the oil was chromatographed on the aged column. These changes in separation of the constituents on the fresh and the aged columns have been used to advantage throughout this study.

The separation of sage oil constituents on the fresh column was necessary to separate myrcene from limonene and partly separate α- and β-thujone. However, the overall separation of sage oil constituents was better on the aged column. In another example the separation of peppermint oil constituents on the aged column was needed for the resolution of 1,8-cineol from peak 13, and menthofuran from peak 29, an unidentified alcohol. However, the overall separation of peppermint oil constituents was better on the fresh column. Thus, two EGS columns of different conditions were used in order to obtain more accurate information about the qualitative and quantitative composition of the essential oils we analyzed.

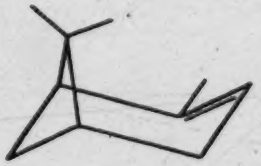
The essential oil constituents can be classified into four groups according to their GLC separation characteristics. Group one is comprised of monoterpene hydrocarbons, p-cymene, a benzene related constituent, and 1,8-cineol, an internal ether. The second group includes the oxygenated monoterpenes which can be divided further into: 1) menthone related constituents, and 2) other oxygenated monoterpenes. Group three is made up of sesquiterpene hydrocarbons and group four is the benzene related constituents.

b. Monoterpene Hydrocarbons, p-Cymene and 1,8-Cineol

The elution sequence of the constituents identified in this group was as follows: α-thujene (b.p. 152°), α-pinene (b.p. 157°), camphene (b.p. 158°-199°), β-pinene (b.p. 164°-166°), α-phellandrene (b.p. 175°), myrcene (b.p. 167°), limonene (b.p. 177°), 1,8-cineol (b.p. 175°), and p-cymene (b.p. 177°-179°). The elution sequence of this group does not strictly follow the boiling points of these constituents and is similar to that of Carbowax 20M. The separation of the group is based on the polarity of the constituents. As Wehrli and Kovats (1959) pointed out, the retention time of these compounds reflects the sum of the increments contributed mainly by the length of carbon skeleton, the degree of chain branching, and the nature and number of the functional groups present. These increments are also attributed to the nature and position along the carbon skeleton of the functional groups and other structural features.



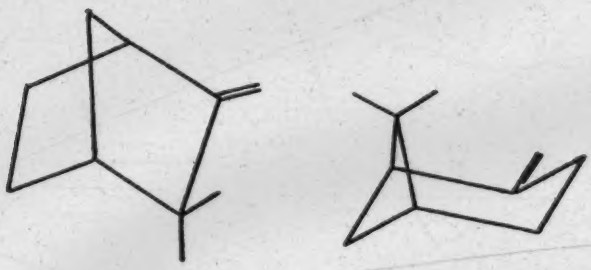
α-Thujene



α-Pinene

α-Thujene was eluted earlier than α-pinene. α-Thujene has an endocyclic double bond as in α-pinene. The polarity of the double bond in α-thujene is greatly reduced by the steric hindrance imparted by its equatorial methyl group. β-Pinene is more polar than α-pinene because it has an exocyclic double bond. This is in accordance with the finding made by Zubyk and Conner (1960), and Klouwen and Heide (1962) that constituents with an exocyclic double bond are more polar than those with

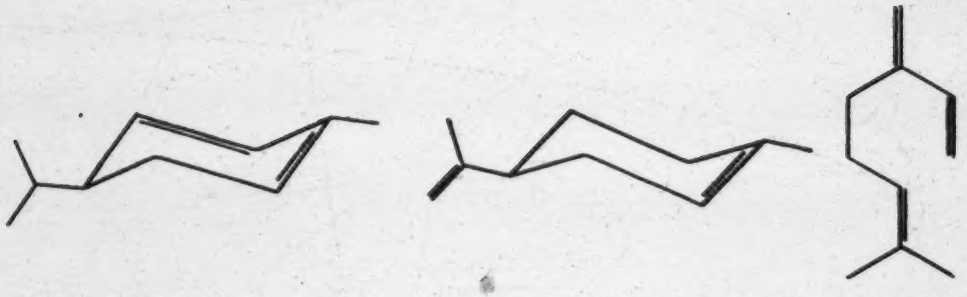
an endocyclic double bond. In addition, the polarity of the double bond in α-pinene is reduced by steric hindrance from the gem-dimethyl group and the cyclobutane ring (Luisetti and Yunes, 1971) as well as from the methyl group. Thus α-pinene emerged earlier than β-pinene in the GLC separation. Camphene, like β-pinene, has an exocyclic double bond, but



Camphene β-Pinene

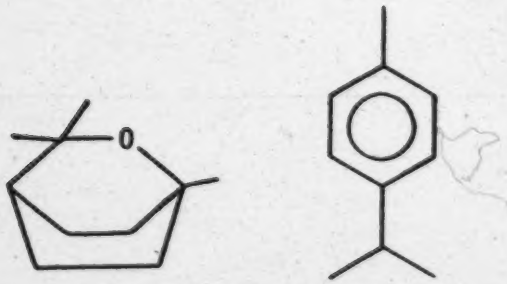
its polarity is less than that of β-pinene because its double bond is hindered by the adjacent gem-dimethyl group. Zubyk and Conner (1960) have found that bicyclic monoterpene hydrocarbons with the second ring formed at the 1,4-positions of the six membered ring, as in camphene, are retained for a longer period than those with the ring formed at the 2,4-positions, as in pinene. However, in our study we found that β-pinene emerged after camphene.

α-Phellandrene is less polar than limonene because its two double bonds are endocyclic, whereas in limonene, one of the double bonds is endocyclic and the other is exocyclic. Consequently, in our separations, α-phellandrene was eluted before limonene. Myrcene is an aliphatic monoterpene hydrocarbon having three double bonds. It was eluted after α-phellandrene but before limonene. This is in agreement



α-Phellandrene Limonene Myrcene

with the findings of Luisetti and Yunes (1971) who established that the polarity is greater for a ring than for a double bond. 1,8-Cineol is an internal ether whose polarity is greatly reduced because of steric hindrance caused by the ring and methyl groups. The polarity of 1,8-cineol is comparable to that of the monoterpene hydrocarbons (Breckler and Betts, 1970). Thus, 1,8-cineol was eluted after limonene. p-Cymene is more polar than limonene because it has a benzene ring.



1,8-Cineol p-Cymene

c. Oxygenated Monoterpenes

i. Menthone Related Constituents

The separation of menthone and menthol stereoisomers was best in the freshly prepared EGS column. On the aged EGS column neomenthol

and neoisomenthol were eluted together as one peak. The results were in accordance with the finding that the separation of isomers could be better achieved on the more polar liquid phases (Cartoni and Liberti, 1960; Bierl et al., 1972). Gillen and Scanlon (1972) found that the limiting factor for the separation of menthone and menthol stereoisomers is the resolution of neoisomenthol either from neomenthol or menthol. The resolution of neoisomenthol depends on the liquid phase selectivity; on 2-hydroxypropyl octakis sucrose (Hyprose SP-80) neomenthol was poorly resolved from neomenthol and on Carbowaxes it tended to elute with menthol. The elution order of menthone related constituents on the EGS column was found by us to be: menthofuran (b.p. 196°), menthone (b.p. 204°), isomenthone (b.p. 208°), neomenthol (b.p. 212°), neoisomenthol (b.p. 214.6°), menthyl acetate (b.p. 227°), menthol (b.p. 216°), isomenthol (b.p. 218°), pulegone (b.p. 224°), and piperitone (b.p. 233°). This elution sequence is different from that on Carbowax 20M and sucrose diacetate hexaisobutyrate (SAIB). In the case of SAIB, menthyl acetate comes out after menthol (Smith and Levi, 1961). The sequence of elution of menthone related constituents on a Carbowax 20M column, as in Table 20, was: menthone, menthofuran, isomenthone, menthyl acetate, neomenthol, neoisomenthyl acetate, menthol, pulegone and piperitone.

Among the menthone related constituents menthofuran is the least polar. It has a furan ring structure with an internal ether and two double bonds. Menthofuran is more polar than 1,8-cineol (also an internal ether) because in addition to a COC group it has two double bonds and, furthermore, the polarity of the COC group is not greatly hindered by the ring structure as it is in 1,8-cineol. The next two constituents which

Menthofuran Menthone Isomenthone

appeared after menthofuran were menthone and isomenthone. Isomenthone is more polar than menthone due to the methyl group being in the axial position in isomenthone and in the equatorial position in menthone (see also Table 21). The methyl group in the equatorial position imparts

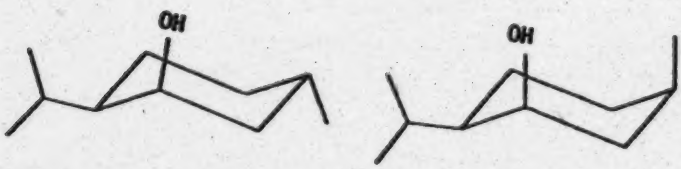
TABLE 21

CONFIGURATION OF MENTHONE AND MENTHOL STEREOISOMERS

Compound	Configuration*		
	Me	iPr	OH
Menthone	e	e	--
Isomenthone	a	e	--
Menthol	e	e	e
Neomenthol	e	e	a
Neoisomenthol	a	e	a
Isomenthol	a	e	e

*e-equatorial; a-axial

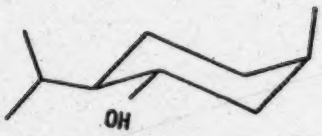
a greater steric hindrance on the polarity of the CO group than that attached in the axial position. Neomenthol and neoisomenthol are more polar than menthone and isomenthone due to the presence of an OH group. Thus, neomenthol and neoisomenthol came out after isomenthone. Of the



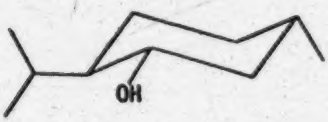
Neomenthol Neoisomenthol

two alcohol constituents, neoisomenthol is more polar than neomenthol due to the methyl group being in the axial position in neoisomenthol and in the equatorial position in neomenthol. Consequently, the cis isomer, neomenthol, was retained by the column for a shorter period of time than trans isomer, neoisomenthol. However, as it is stated earlier, due to conformation equilibria the polarities of these alcohols might be disputed.

Menthol and isomenthol are more polar than neomenthol and neoisomenthol because of the position of their OH group. The isopropyl group, which is in equatorial position in all menthol stereoisomers, imparts a greater steric hindrance to the polarity of an axial OH group, as in neomenthol and neoisomenthol, than to an equatorial OH group, as



Isomenthol

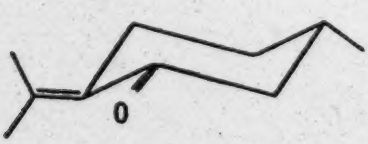


Menthol

in menthol and isomenthol. Of the two constituents, menthol is less polar than isomenthol because it has an equatorial methyl group. Menthyl acetate was eluted before menthol. This was due to the polarity

Menthyl acetate

of menthyl acetate being less than that of menthol because of the esterified OH group. The last two constituents, which emerged after isomenthol, were pulegone and piperitone. These two constituents are more polar than menthone and menthol stereoisomers because of their CO group and double bond. The double bond is endocyclic in piperitone and exocyclic in pulegone. However, piperitone is more polar than pulegone



Pulegone

Piperitone

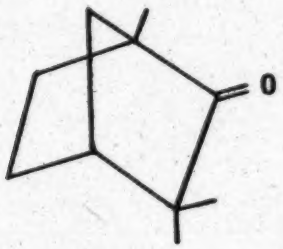
because the polarity of the double bond in pulegone is hindered by methyl groups.

ii. Other Oxygenated Monoterpenes

The overall separation of these oxygenated monoterpenex was best achieved using the aged EGS column. However, the use of the fresh EGS column was necessary to separate isomers such as α- and β-thujone. This is in agreement with the finding that the separation of isomers is best on the more polar liquid phases (Cartoni and Liberti, 1960; Bierl et al., 1972). The separation on the fresh column showed that camphor and linalool were eluted as one peak. The elution sequence of these oxygenated monoterpenes on the aged column was: fenchone (b.p. 193°), α-thujone (b.p. 201°), β-thujone, camphor (b.p. 208°), linalool (b.p. 198°), bornyl acetate (b.p. 208°), dihydrocarvyl acetate (b.p. 272°), borneol (b.p. 208°), α-terpineol (b.p. 219°) and carvone (b.p. 230°). This elution sequence is slightly different from that on a Carbowax 20M column. The elution order of these oxygenated monoterpenes on Carbowax 20M was: fenchone and α-thujone as one peak, β-thujone, linalool and camphor as one peak, linalyl acetate, bornyl acetate, borneol and α-terpineol (Lawrence et al., 1971).

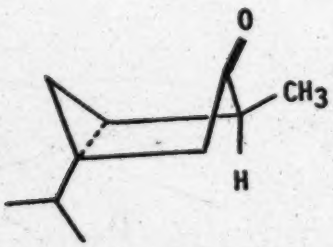
The polarity of these compounds depends on their functional group or groups, the number and position of double bonds, the configuration, the number of rings and other atoms or groups that can cause steric hindrance to the polarized zones. The retention of the individual components in the column is also determined by their molecular weight. The steric hindrance caused by the adjacent gem-dimethyl group on the

polarity of the CO group in fenchone is greater than that imparted by the methyl group on the polarity of the CO group in thujone stereoisomers. Consequently, fenchone was eluted earlier than α- and β-thujone. Of the two stereoisomers, β-thujone is more polar than α-thujone because

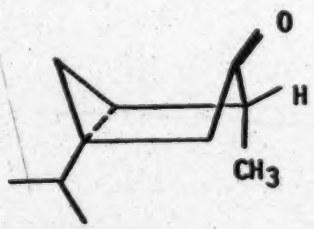


Fenchone

of the position of the methyl group which is axial in β-thujone and equatorial in α-thujone. The methyl group in the equatorial position is closer to the CO group than the methyl group in the axial position. Hence, the steric hindrance imparted by the methyl group in the equatorial position on the polarity of the CO group is greater than that caused by the methyl group in the axial position.

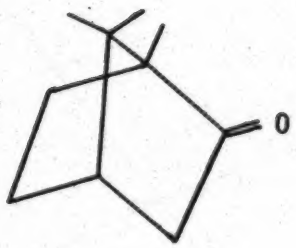


α-Thujone



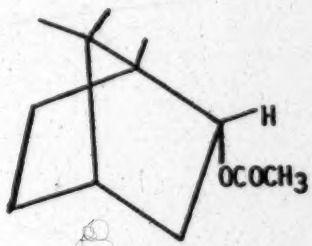
β-Thujone

The component which emerged after β-thujone was camphor. The polarity of the CO group is much less hindered by the gem-dimethyl group in camphor than by the methyl group in thujone. This is because the methyl group in the thujone stereoisomers is closer to the CO group. Linalool, a tertiary alcohol, was eluted after camphor. The polarity of the OH group in linalool is greatly hindered by the methyl and vinyl groups. However, the polarity of linalool is still greater than camphor because of the two double bonds. Bornyl acetate appeared after linalool.



Camphor

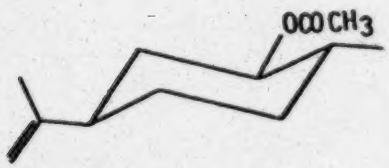
Linalool



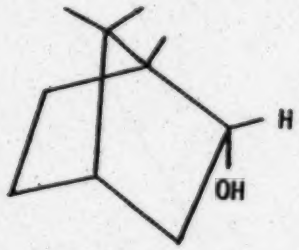
Bornyl acetate

Bornyl acetate had a higher retention time than linalool due to the polarity of the $OCOCH_3$ group and its molecular weight (linalool, 154; bornyl acetate, 196). The polarity of the axial $OCOCH_3$ group is not hindered greatly by the adjacent methyl and gem-dimethyl groups. The component that appeared after bornyl acetate was dihydrocarvyl acetate. A longer retention of dihydrocarvyl acetate than bornyl acetate is explained by the polarity of the $OCOCH_3$ group, and the presence of a double bond.

In all GLC runs borneol was retained longer than dihydrocarvyl acetate. This is explained by the polarity of the borneol OH group, which is less hindered by the methyl and gem-dimethyl groups. α-Terpineol,



Dihydrocarvyl acetate



Borneol

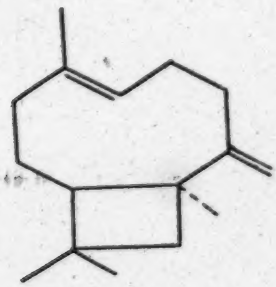
a tertiary alcohol, was eluted after borneol. Besides the OH group, α-terpineol has an endocyclic double bond. Although both α-terpineol and linalool are tertiary alcohols the former is more polar than the latter because it has a cyclohexane ring. As Luisetti and Yunes (1971) established, the polarity is greater for rings than for double bonds because the rings involve the whole molecule while the double bonds involve only a part of the molecule. Though the polarity of the OH group is greatly hindered by the two methyl groups, the retention of α-terpineol is still longer than that of borneol because of its double bond. Finally, carvone was eluted after α-terpineol. The polarity of carvone is attributed to the CO group and to the two double bonds. Further, the endocyclic double bond is conjugated with the CO group. However, the polarity of the endocyclic double bond and the CO group is slightly hindered by the adjacent methyl group.

α-Terpineol

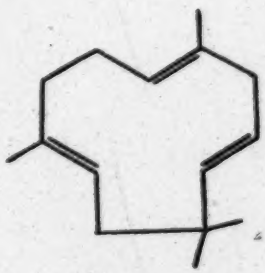
Carvone

d. Sesquiterpene Hydrocarbons

The two sesquiterpene hydrocarbons identified in this study were β-caryophyllene (b.p. 256°) and humulene (α-caryophyllene). These sesquiterpene hydrocarbons were eluted in the same region as the oxygenated monoterpenes. β-Caryophyllene appeared after bornyl acetate and humulene after borneol. The high retention times of the sesquiterpene hydrocarbons were mainly due to their high molecular weight, which is 204 for both sesquiterpenes, and also to their double bonds. During GLC separations β-caryophyllene had a lower retention time than humulene. Humulene is more polar than β-caryophyllene because it has three double bonds while β-caryophyllene has only two. The exocyclic



β-Caryophyllene



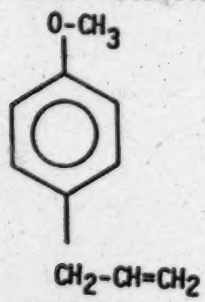
Humulene
(α-Caryophyllene)

double bond of β-caryophyllene is shielded by the cyclobutane methylene group while the non-methylated double bond in humulene is shielded by the gem-dimethyl group (Luisetti and Yunes, 1971).

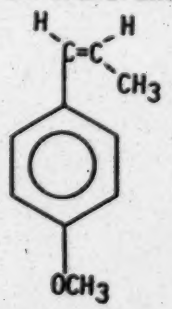
e. Benzene Related Components

The separation of the benzene related constituents in essential oils was achieved equally well on both fresh and aged columns. In general, this group of compounds is more polar than the oxygenated monoterpenes due to its basic structure of a benzene ring and to the presence of a methoxy group. However, out of these constituents, estragole, cis-anethole, and trans-anethole were eluted among the oxygenated monoterpenes. With the EGS column, the elution sequence of the benzene derivative constituents was: estragole (b.p. 216°), cis-anethole, trans-anethole (b.p. 236°), p-anisic acid (b.p. 275-280°), p-anisaldehyde (b.p. 248°), anisyl alcohol (b.p. 259°), eugenol (b.p. 253°) and anisyl acetone.

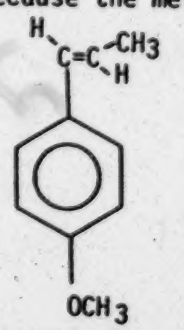
In addition to the basic structure, estragole, cis-anethole and trans-anethole each have a double bond. However, estragole was found to be less polar than the anethole isomers because it has an isolated double bond whereas the anethole isomers have their double bond conjugated with the benzene ring. Of the two anethole isomers, cis-anethole had a lower retention time than trans-anethole because the methyl group



Estragole



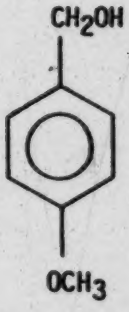
cis-Anethole



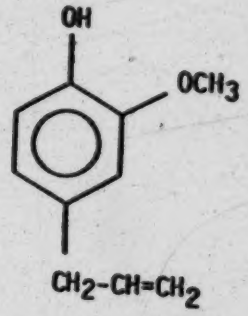
trans-Anethole

in the cis-position in anethole imparts a greater steric hindrance to the polarity of the benzene ring than the same group in the trans-position. p-Anisaldehyde was eluted earlier than anisyl alcohol because the former has, besides the basic structure, a CHO group whereas the latter has an OH group. On the other hand, eugenol was retained for a longer period of time than anisyl alcohol, because, in addition to the

p-Anisaldehyde

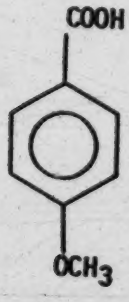


Anisyl alcohol

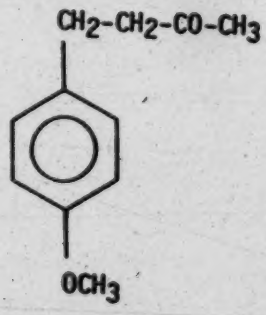


Eugenol

basic structure, it has an OH and a double bond. Anisyl acetone was retained for the longest period of time among this group of compounds.



p-Anisic acid



Anisyl acetone

Besides the basic structure, anisyl acetone has a CO group which is less polar than an OH group. However, the factor which further contributes to its retention in the column is its molecular weight (anisyl acetone, 178; eugenol, 164). Finally, p-anisic acid appeared between trans-anethole and p-anisaldehyde, even though, besides the basic structure, it contains a COOH group which is known to be more polar than the CHO and OH groups. This fact indicates that the EGS column is less selective to the COOH group.

2. Individual Essential Oils

a. Anise Seed Oil

The GLC of anise seed oil from Brooks--1971 crop, Fig. 15, shows the separation of the components on the aged column. The peaks identified in the chromatogram of this oil were: α-pinene (1), α-phellandrene (4), myrcene (5), limonene (6), fenchone (14), camphor (18), linalool (20), β-caryophyllene (28), estragole (29), dihydrocarvyl acetate (33), cis-anethole (34), carvone (35), trans-anethole (38), p-anisic acid (44), anisyl alcohol (46), eugenol (51) and anisyl acetone (64). The major peak was 38 (trans-anethole) and the medium peaks were 6 (limonene), 29 (estragole) and peaks 32, 37 and 63. Myrcene (5) and limonene (6) were not resolved, nor β-caryophyllene (28) and estragole (29). Peak 7 was slightly separated from limonene (6). Peaks 32, 33 (dihydrocarvyl acetate), 34 (cis-anethole), 35 (carvone), 36, 37 and 38 (trans-anethole) were bunched closely together. The separation of the constituents of anise oil from Michigan on the aged column is illustrated in Fig. 16. In this oil the medium peaks were peak numbers 6 (limonene) and 44 (p-anisic acid). Peaks 63 and 64 were not found in Michigan oil. The

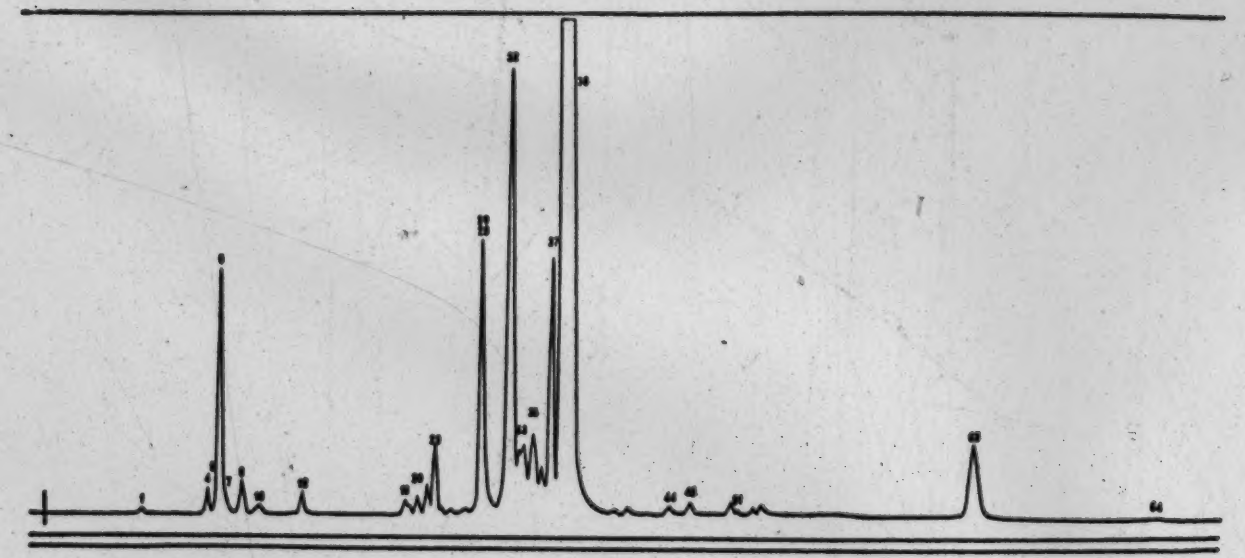


Fig. 15. Gas-Liquid Chromatogram of Anise Seed Oil on the Aged Column (Brooks--1971 Crop).

Gas Chromatograph: Bendix M 2500 equipped with flame ionization detectors.
Column: U-shaped glass 6'x1/8"I.D. packed with 15% EGS on Chromosorb P, AW, 100/120 mesh.
Temperature: Programmed 50-195°C at 4°C/min.
Carrier Gas: N_2 60 ml/min; Chart Speed: 60 cm/hr; Sample Volume Injected: 1 µl.

Peaks identified: (1)α-pinene, (4)α-phellandrene, (5) myrcene, (6) limonene, (14) fenchone, (18) camphor, (20) linalool, (28)β-caryophyllene, (29) estragole, (33) dihydrocarvyl acetate, (34) cis-anethole, (35) carvone, (38) trans-anethole, (44) p-anisic acid, (46) anisyl alcohol, (51) eugenol and (64) anisyl acetone.

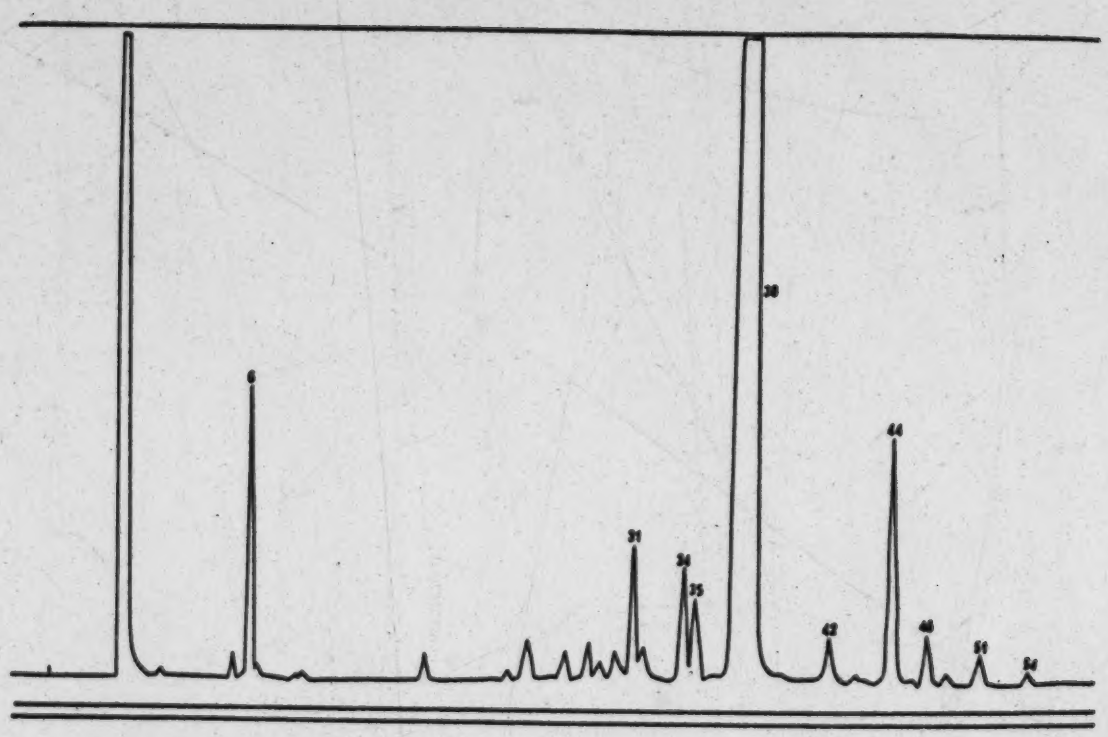


Fig. 16. Gas-Liquid Chromatogram of Anise Seed Oil on the Aged Column (Michigan).

Peaks identified: (1)α-pinene, (4)α-phellandrene, (5) myrcene, (6) limonene, (14) fenchone, (18) camphor, (20) linalool, (28) β-caryophyllene, (29) estragole, (33) dihydrocarvyl acetate, (34) cis-anethole, (35) carvone, (38) trans-anethole, (44) p-anisic acid, (46) anisyl alcohol, (51) eugenol and (64) anisyl acetone

peaks separated on the aged column were sharper in shape than those separated on the fresh column.

The chromatogram of anise oil from Brooks--1971 crop (Fig. 17) shows the separation of the constituents on the fresh column. The sequence of elution of the components was different from that of the aged column. Myrcene (5) was separated from limonene (6), and β-caryophyllene (28) from estragole (29). However, estragole (29) and dihydrocarvyl acetate (33) were eluted as one peak and were not well separated from peak 37. Peak 7 was eluted together with peak 6 (limonene). Cis-anethole (34), carvone (35), peak 36 and trans-anethole (38) were well separated on this column. It took a longer time to run the complete chromatogram on the fresh column than on the aged column.

b. Caraway Seed Oil

The GLC separation of caraway seed oil from Brooks--1971 crop on the aged column is given in Fig. 18 and the separation on the fresh column in Fig. 19. The peaks identified in the chromatograms of caraway oils were: α-pinene (2), β-pinene (5), α-phellandrene (9), myrcene (10), limonene (11), α-thujone (23), β-thujone (24), camphor (26), linalool (28), β-caryophyllene (36), carvone (42) and trans-anethole (44). There were two major peaks, peak numbers 11 (limonene) and 42 (carvone) and one medium peak, 10 (myrcene). Peak 10 (myrcene) was separated from peak 11 (limonene) on the fresh column. Peak 43 was partially separated from peak 42 (carvone) on the aged column. Peak 9 (α-phellandrene) was well resolved from peak 11 (limonene) on the aged column. β-Caryophyllene (36) was well separated from peak 37 on the fresh column.

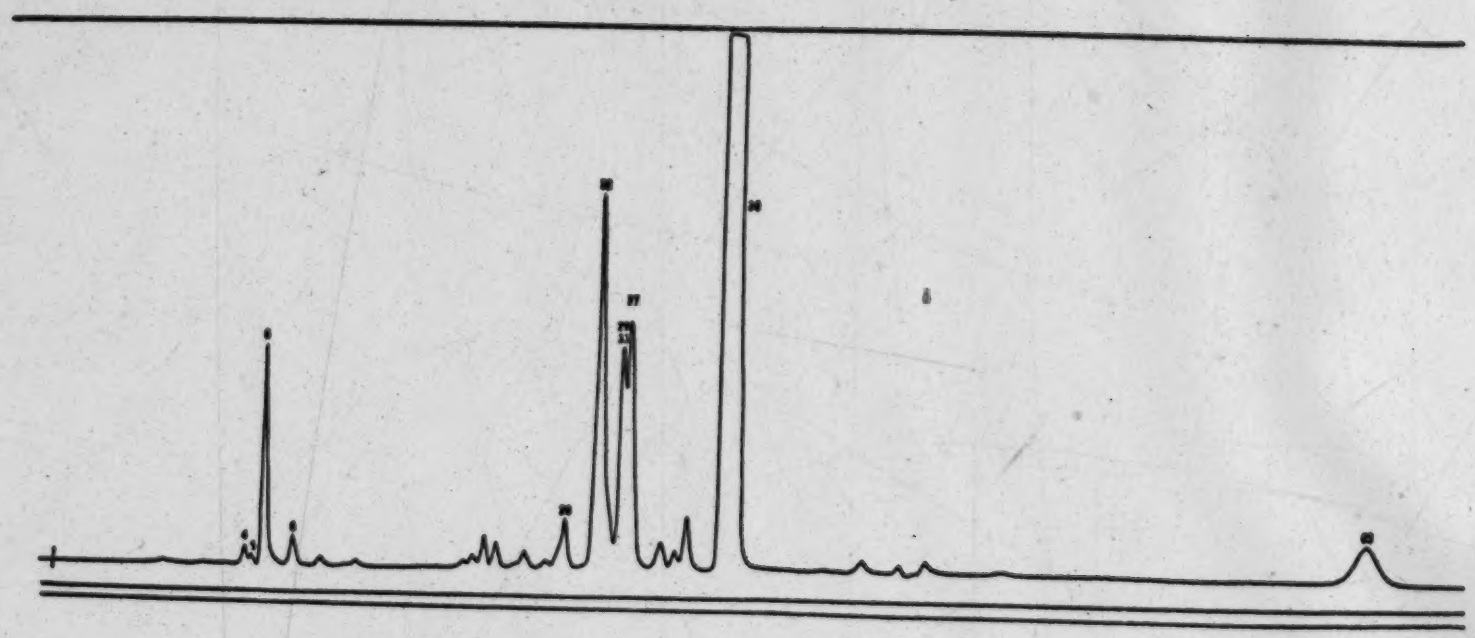


Fig. 17. Gas-Liquid Chromatogram of Anise Seed Oil on the Fresh Column (Brooks--1971 Crop).

Peaks identified: (1)α-pinene, (4)α-phellandrene, (5) myrcene, (6) limonene, (14) fenchone, (18) camphor, (20) linalool, (28) β-caryophyllene, (29) estragole, (33) dihydrocarvyl acetate, (34) cis-anethole, (35) carvone, (38) trans-anethole, (44) p-anisic acid, (46) anisyl alcohol, (51) eugenol and (64) anisyl acetone.

Fig. 18. -Gas-Liquid Chromatogram of Caraway Seed Oil on the Fresh Column (Brooks--1971 Crop).

Gas Chromatograph: Bendix M 2500 with flame ionization detectors.
Column: U-shaped glass 6'x1/8"I.D. packed with 15% EGS on Chromosorb P, AW, 100/120 mesh.
Temperature: Programmed 50-195°C at 4°C/min.
Carrier Gas: N_2 60 ml/min; Chart Speed: 60 cm/hr;
Sample Volume Injected: 1 µl.

Peaks identified: (2)α-pinene, (5)β-pinene, (9)α-phellandrene, (10) myrcene, (11) limonene, (23)α-thujone, (24)β-thujone, (26) camphor, (28) linalool, (36)β-caryophyllene, (42) carvone, (44) trans-anethole.

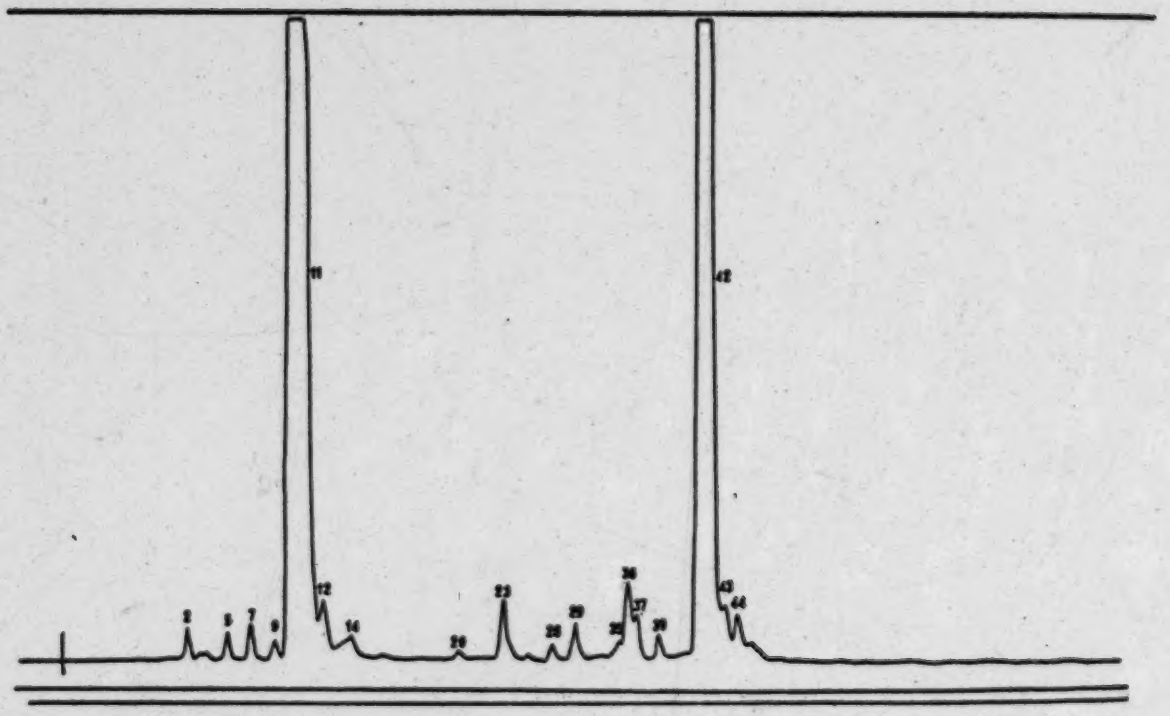


Fig. 19. Gas-Liquid Chromatogram of Caraway Seed Oil on the Aged Column (Brooks--1971 Crop).

Peaks identified: (2)α-pinene, (5)β-pinene, (9)α-phellandrene, (10) myrcene, (11) limonene, (23)α-thujone, (24)β-thujone, (26) camphor, (28) linalool, (36)β-caryophyllene, (42) carvone, (44) trans-anethole.

c. Dill Oil

The chromatogram of Danish dill seed oil from Brooks--1971 crop (Fig. 20) shows the separation of the constituents on the fresh column. The chromatogram of the constituents of dill prime oil from Michigan on the aged column is illustrated in Fig. 21. The peaks identified in the chromatograms of dill oils were: α-pinene (1), camphene (2), β-pinene (3), α-phellandrene (6), myrcene (7), limonene (8), p-cymene (12), carvone (31) and trans-anethole (35). The chromatograms of Danish and common dill seed oils from Brooks, showed three major peaks, 6 (α-phellandrene), 8 (limonene), and 31 (carvone), while that of dill prime and standard from Michigan revealed five major peaks: 6 (α-phellandrene), 8 (limonene), 9, 20 and 31 (carvone). The medium peaks shown on the GLC of Danish and common dill seed oil were: 10 (1,8-cineol), 20 and 27 while the medium peaks revealed on GLC of dill prime and standard oil were: 1 (α-pinene), 12 (p-cymene) and 27. As usual, myrcene (7) was separated from limonene (8) on the fresh column.

d. Fennel Seed Oil

The GLC separation on the fresh column of the constituents of fennel seed oil from Brooks--1971 crop, is given in Fig. 22. The fresh column GLC separation of the constituents of commercial fennel seed oil from Fritzsche is illustrated in Fig. 23. The peaks identified in the chromatogram of this oil were: α-pinene (4), camphene (7), β-pinene (8), α-phellandrene (11), myrcene (12), limonene (13), 1,8-cineol (14), p-cymene (18), fenchone (27), α-thujone (29), camphor (31), borneol (41), estragole (42), cis-anethole (45), carvone (46), trans-anethole (49), p-anisic acid (58), anisyl alcohol (59) and eugenol (62). The

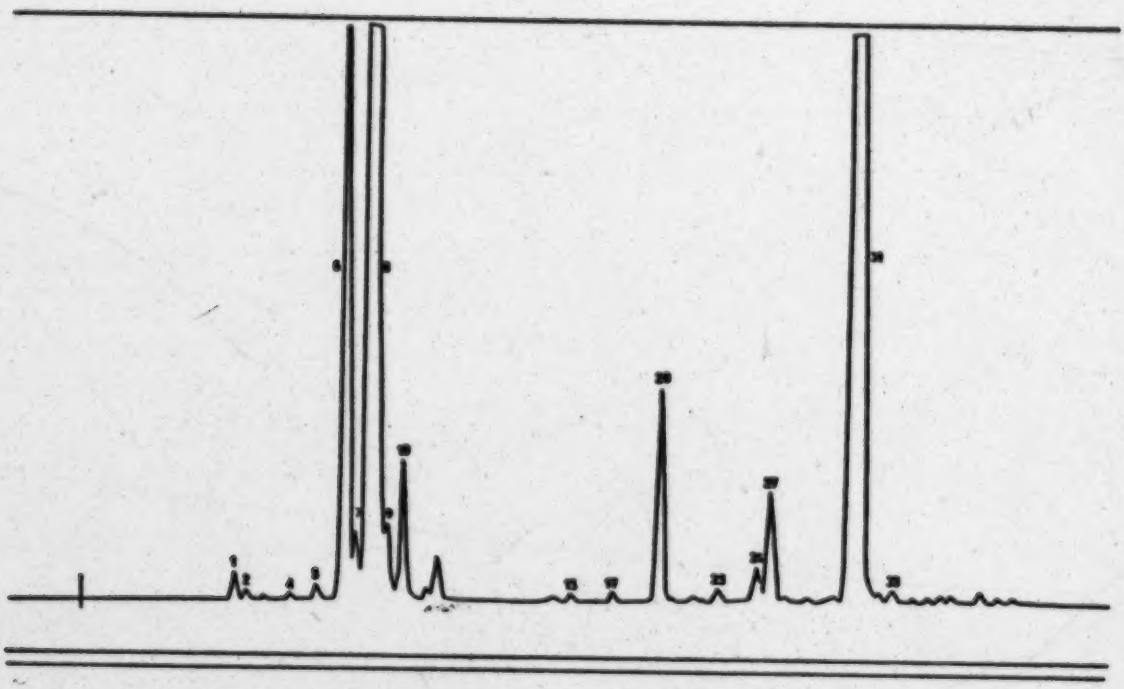


Fig. 20. Gas-Liquid Chromatogram of Danish Dill Seed Oil on the Fresh Column (Brooks--1971 Crop).

Gas Chromatograph: Bendix M 2500 equipped with flame ionization detectors.
Column: U-shaped glass 6'x1/8"I.D. packed with 15% EGS on Chromosorb P, AW, 100/120 mesh.
Temperature: Programmed 50-195°C at 4°C/min.
Carrier Gas: N_2 60 ml/min; Chart Speed: 60 cm/hr;
Sample Volume Injected: 1 µl.

Peaks identified: (1)α-pinene, (2) camphene, (3)β-pinene, (6)α-phellandrene, (7) myrcene, (8) limonene, (9) 1,8-cineol, (12) p-cymene, (31) carvone, (35) trans-anethole.

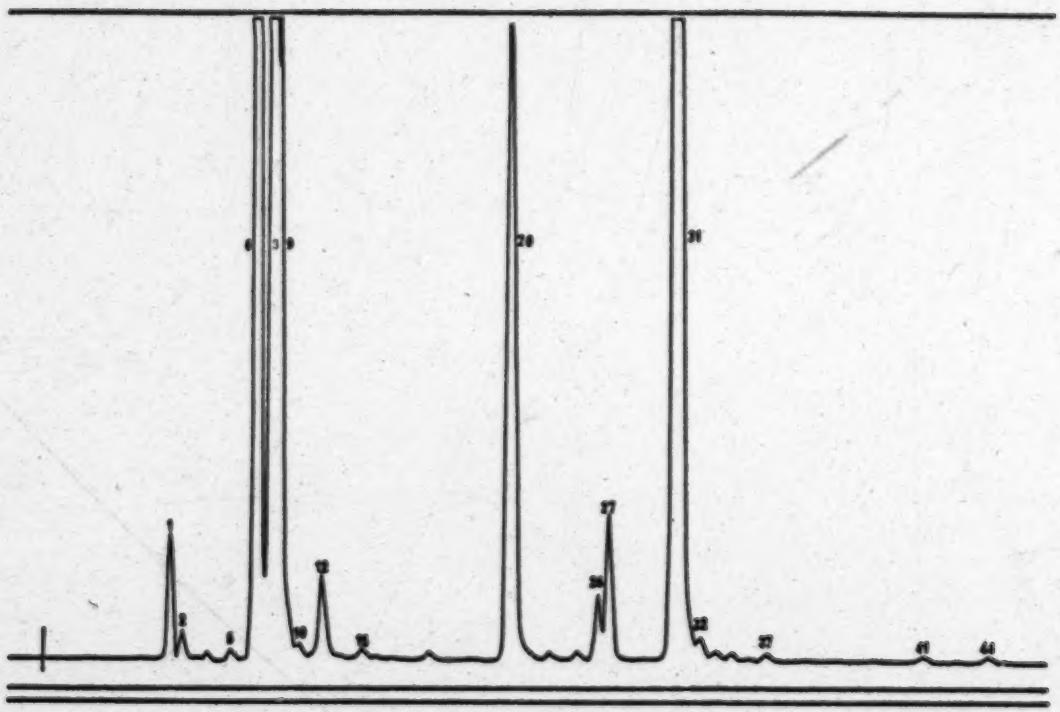


Fig. 21. Gas-Liquid Chromatogram of Dill Prime Oil on the Aged Column (Michigan)

Peaks identified: (1)α-pinene, (2) camphene, (3)β-pinene, (6)α-phellandrene, (7) myrcene, (8) limonene, (9) 1,8-cineol, (12) p-cymene, (31) carvone, (35) trans-anethole.

Fig. 22. Gas-Liquid Chromatogram of Fennel Seed Oil on the Fresh Column (Brooks--1971 Crop).

Gas Chromatograph: Bendix M 2500 equipped with flame ionization detectors.
Column: U-shaped glass 6'x1/8"I.D. packed with 15% EGS on Chromosorb P, AW, 100/120 mesh.
Temperature: Programmed 50-195°C at 4°C/min.
Carrier Gas: N_2 60 ml/min; Chart Speed: 60 cm/hr;
Sample Volume Injected: 1 µl.

Peaks identified: (4)α-pinene, (7) camphene, (8)β-pinene, (11)α-phellandrene, (12) myrcene, (13) limonene, (14) 1,8-cineol, (18) p-cymene, (27) fenchone, (29)α-thujone, (31) camphor, (41) borneol, (42) estragole, (45) cis-anethole, (46) carvone, (49) trans-anethole, (58) p-anisic acid, (59) anisyl alcohol, (62) eugenol.

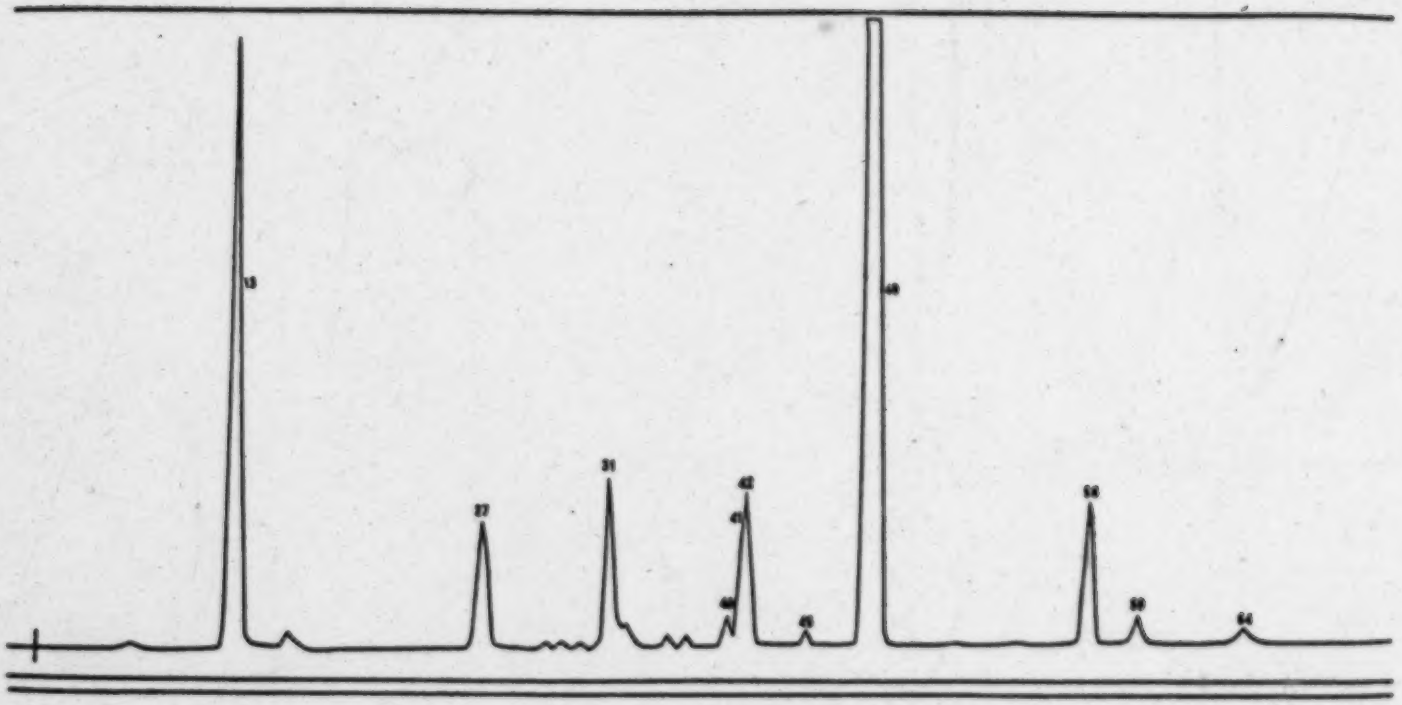


Fig. 23. Gas-Liquid Chromatogram of Fennel Seed Oil on the Fresh Column (Fritzsche).

Peaks identified: (4)α-pinene, (7) camphene, (8)β-pinene, (11)α-phellandrene, (12) myrcene, (13) limonene, (14) 1,8-cineol, (18) p-cymene, (27) fenchone, (29)α-thujone, (31) camphor, (41) borneol, (42) estragole, (45) cis-anethole, (46) carvone, (49) trans-anethole, (58) p-anisic acid, (59) anisyl alcohol, (62) eugenol.

chromatograms of fennel seed oil from Brooks--1971 and 1972 crops showed three major peaks, limonene (13), fenchone (27) and trans-anethole (49) while that of fennel oil from Fritzsche revealed two major peaks, limonene (13) and trans-anethole (49). The medium peaks shown on the GLC of fennel seed oil from Brooks--1971 and 1972 crops were: α-pinene (4), myrcene (12), 17, camphor (31) and estragole (42) while the medium peaks identified in the chromatogram of fennel seed oil from Fritzsche were: fenchone (27), camphor (31), borneol (41), estragole (42) and p-anisic acid (58). The separation of myrcene (12) and limonene (13) in fennel seed oil was achieved on the fresh column but not on the aged column.

e. Peppermint Oil

The GLC separation of peppermint oil from Michigan, Fig. 24, shows the separation of the constituents on the fresh column. The separation conditions were: temperature programmed from 60° to 150° at 1°/min and N_2 flow rate, 30 ml/min. The GLC separation of the constituents of peppermint oil from Brooks--1970 crop under changed separation conditions on the fresh column is illustrated in Fig. 25, and on the aged column in Fig. 26. The separation conditions of these two chromatograms were: temperature programmed from 50° to 195° at 4°/min and N_2 flow rate 60 ml/min. The peaks identified in the peppermint oil chromatograms were: α-pinene (1), camphene (2), β-pinene (4), α-phellandrene (8), myrcene (9), limonene (10), 1,8-cineol (12), p-cymene (16), 3-octanol (24), menthofuran (28), menthone (31), isomenthone (34), linalool (36), neomenthol (37), β-caryophyllene (38), menthyl acetate (39), neoisomenthol (40), menthol (42), isomenthol (43), pulegone (47) and piperitone (54). The GLC separation of the constituents of peppermint oils from Brooks,

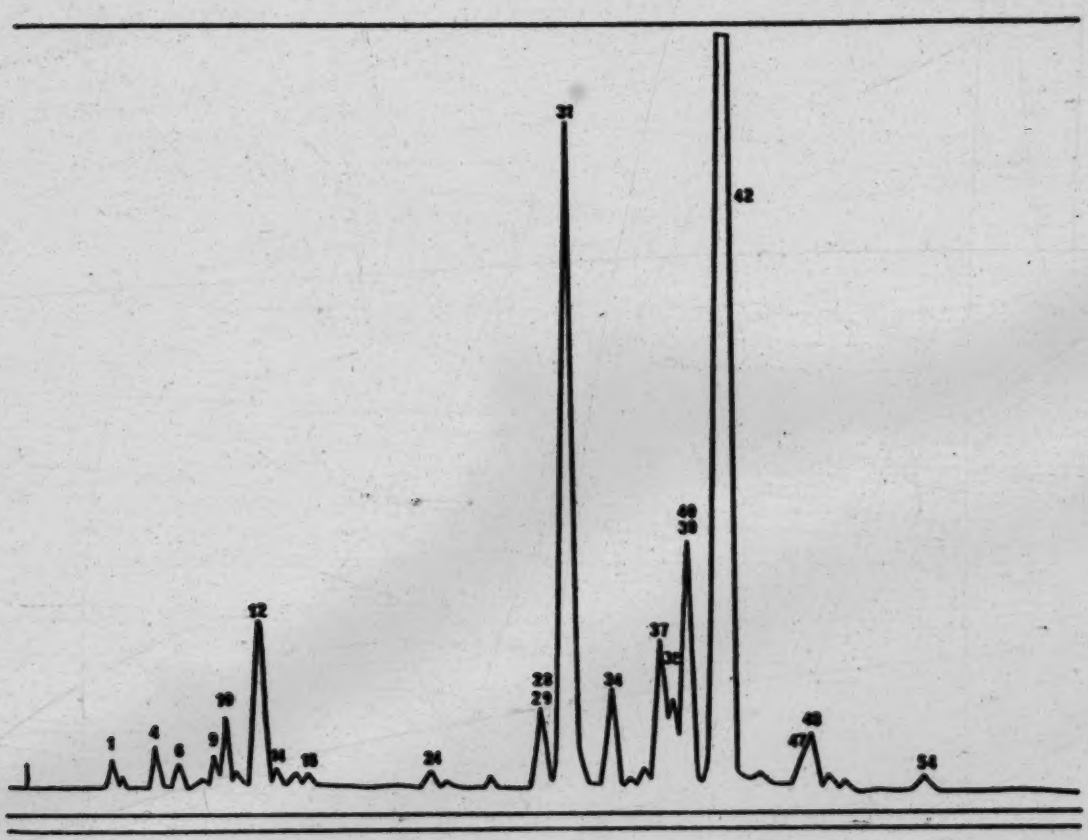


Fig. 24. Gas-Liquid Chromatogram of Peppermint Oil on the Fresh Column (Michigan).

Gas Chromatograph: Bendix M 2500 equipped with flame ionization detectors.
Column: U-shaped glass 6'x1/8"I.D. packed with 15% EGS on Chromosorb P, AW, 100/120 mesh.
Temperature: Programmed 60-150°C at 1°C/min.
Carrier Gas: N_2 30 ml/min; Chart Speed: 20 cm/hr;
Sample Volume Injected: 1 µl.

Peaks identified: (1)α-pinene, (2) camphene, (4)β-pinene, (8)α-phellandrene, (9) myrcene, (10) limonene, (12) 1,8-cineol, (16) p-cymene, (24) 3-octanol, (28) menthofuran, (31) menthone, (34) isomenthone, (36) linalool, (37) neo-menthol, (38)β-caryophyllene, (39) menthyl acetate, (40) neoisomenthol, (42) menthol, (43) isomenthol, (47) pulegone, (54) piperitone.

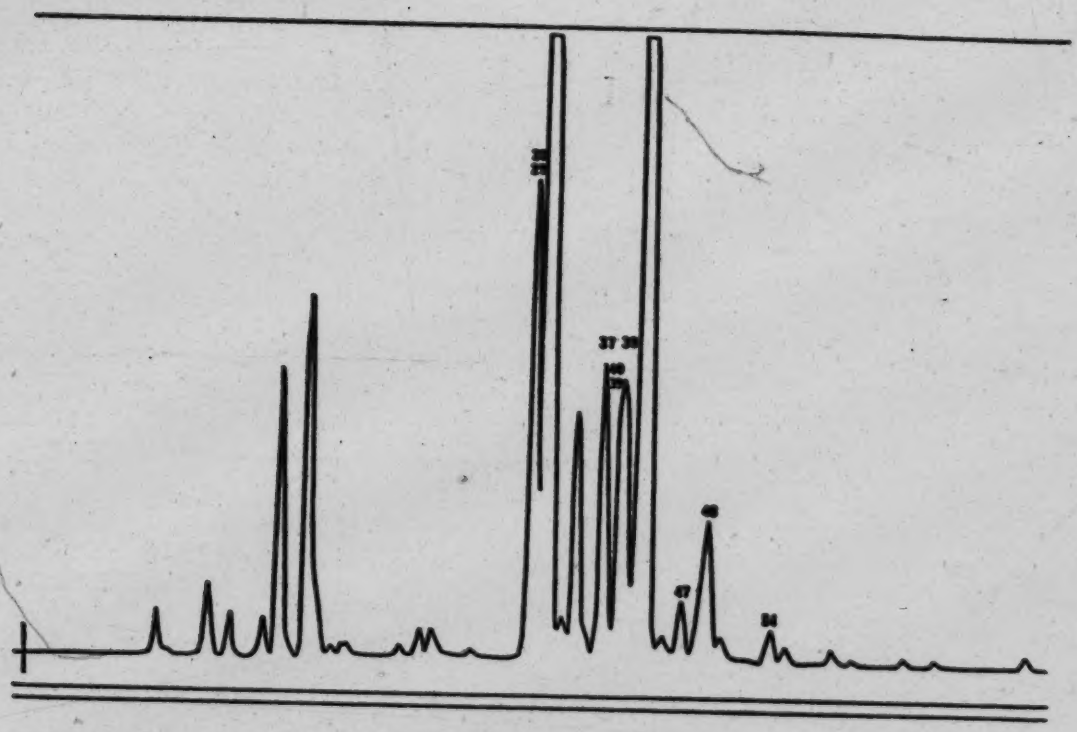


Fig. 25. Gas-Liquid Chromatogram of Peppermint Oil on the Fresh Column (Brooks--1970 Crop).

Temperature: Programmed 50-195°C at 4°C/min.
Carrier Gas: N_2 60 ml/min; Chart Speed: 60 cm/hr;
Sample Volume Injected: 1 µl.

Peaks identified: (1)α-pinene, (2) camphene, (4)β-pinene, (8)α-phellandrene, (9) myrcene, (10) limonene, (12) 1,8-cineol, (16) p-cymene, (24) 3-octanol, (28) menthofuran, (31) menthone, (34) isomenthone, (36) linalool, (37) neo-menthol, (38)β-caryophyllene, (39) menthyl acetate, (40) neoisomenthol, (42) menthol, (43) isomenthol, (47) pulegone, (54) piperitone.

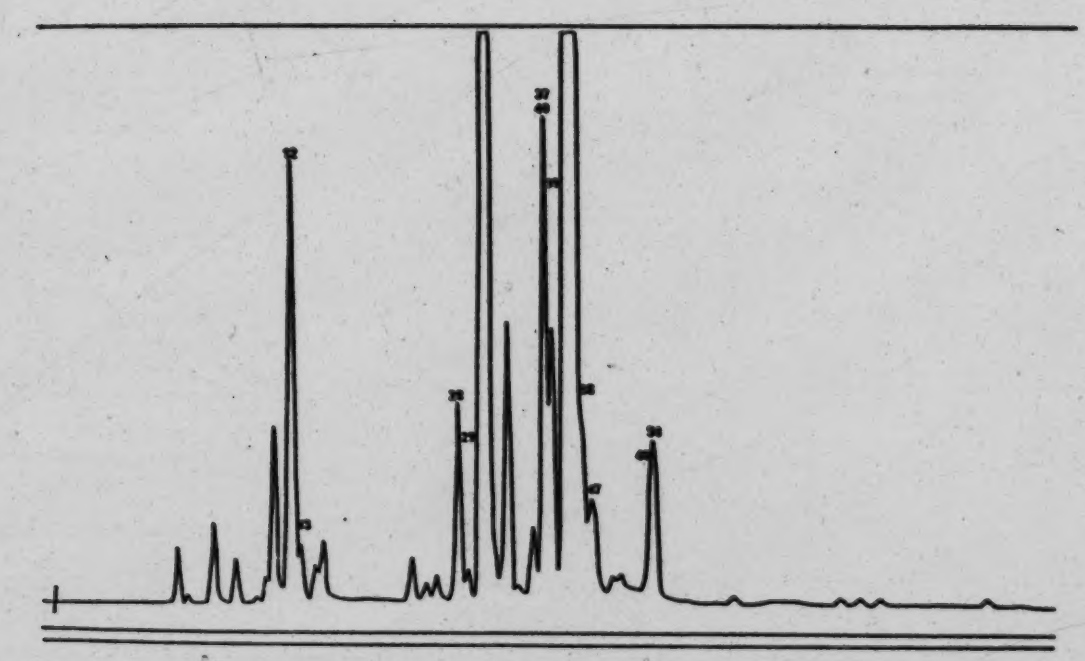


Fig. 26. Gas-Liquid Chromatogram of Peppermint Oil on the Aged Column (Brooks--1970 Crop).

Temperature: Programmed 50-195°C at 4°C/min.
Carrier Gas: N_2 60 ml/min; Chart Speed: 60 cm/hr;
Sample Volume Injected: 1 µl.

Peaks identified: (1)α-pinene, (2) camphene, (4)β-pinene, (8)α-phellandrene, (9) myrcene, (10) limonene, (12) 1,8-cineol, (16) p-cymene, (24) 3-octanol, (28) menthofuran, (31) menthone, (34) isomenthone, (36) linalool, (37) neomenthol, (38)β-caryophyllene, (39) menthyl acetate, (40) neoisomenthol, (42) menthol, (43) isomenthol, (47) pulegone, (54) piperitone.

Beaverlodge and Michigan showed two major peaks, menthone (31) and menthol (42). Seven medium peaks found on GLC of the Brooks and Michigan oils were: limonene (10), 1,8-cineol (12), menthofuran (28), isomenthone (34), neomenthol (37), β-caryophyllene (38) and menthyl acetate (39). Menthofuran was not a medium peak in Beaverlodge oils.

The chromatogram of peppermint oil constituents under conditions given in Fig. 24 showed that myrcene (9) was resolved from limonene (10). Peak 12 (1,8-cineol) was eluted together with peak 13, peak 28 (menthofuran) with 29 (an alcohol), peak 39 (menthyl acetate) with 40 (neoisomenthol), and peak 47 (pulegone) with 48. The GLC separation of peppermint oil constituents as presented in Fig. 25 was slightly different from that of Fig. 24. In this separation, peaks 28 (menthofuran) and 31 (menthone) were not well resolved, peak 38 (β-caryophyllene) was eluted after menthyl acetate and neoisomenthol and, in addition, the peaks were not resolved. Peak 47 (pulegone) was well separated from peak 48. The chromatogram of peppermint oil on the aged column (see Fig. 26) showed that peak 12 (1,8-cineol) was partly resolved from peak 13. In addition, menthofuran (28) was separated from peak 29 (an alcohol); neomenthol (37) and neoisomenthol (40) were eluted together as one peak; peak 38 (β-caryophyllene) came out after peak 42 (menthol) and both peaks were not resolved; pulegone (47) appeared after β-caryophyllene (38) and peak 48 was eluted together with piperitone (54).

f. Sage Oil

A fresh column GLC separation of the constituents of sage oil from Kalamazoo, Michigan, is given in Fig. 27, and that of sage oil from Brooks--1971 crop in Fig. 28. The chromatogram on the aged column of

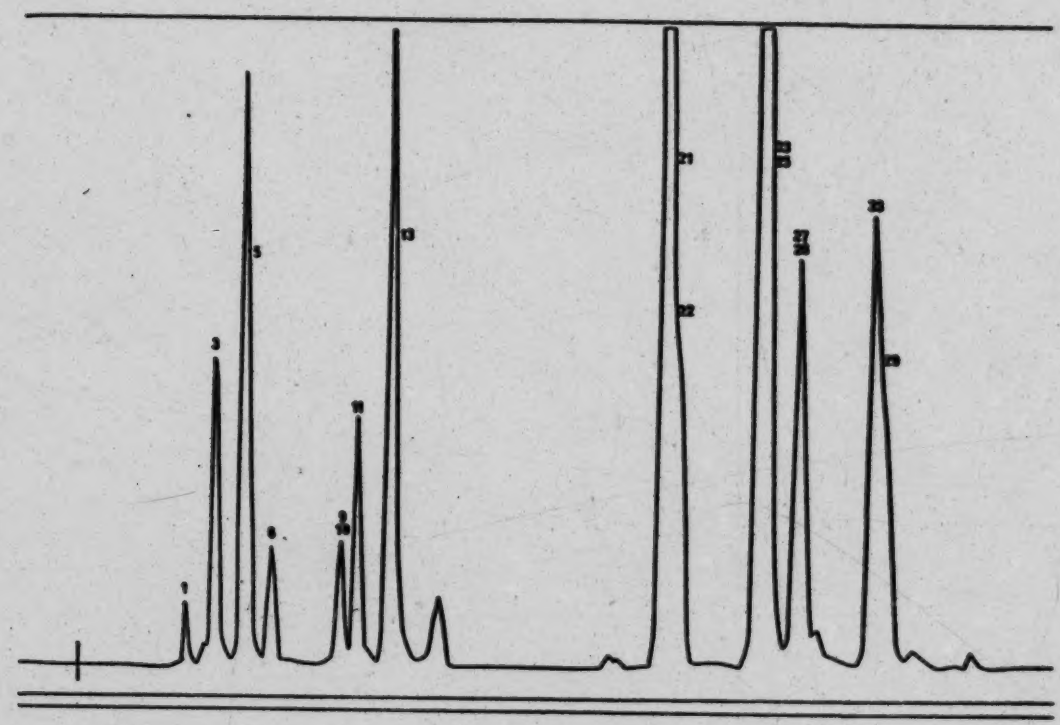


Fig. 27. Gas-Liquid Chromatogram of Sage Oil on the Fresh Column (Michigan).

Gas Chromatograph: Bendix M 2500 equipped with flame ionization detectors.
Column: U-shaped glass 6'x1/8"I.D. packed with 15% EGS on Chromosorb P, AW, 100/120 mesh.
Temperature: Programmed 50-195°C at 4°C/min.
Carrier Gas: N_2 60 ml/min; Chart Speed: 60 cm/hr;
Sample Size Injected: 1 µl.

Peaks identified: (1)α-thujene, (3)α-pinene, (5) camphene, (6)β-pinene, (8)α-phellandrene, (10) myrcene, (11) limonene, (13) 1,8-cineol, (17) p-cymene, (19) fenchone, (21)α-thujone, (22)β-thujone, (23) camphor, (25) linalool, (27) bornyl acetate, (28)β-caryophyllene, (29) borneol, (33) humulene (α-caryophyllene).

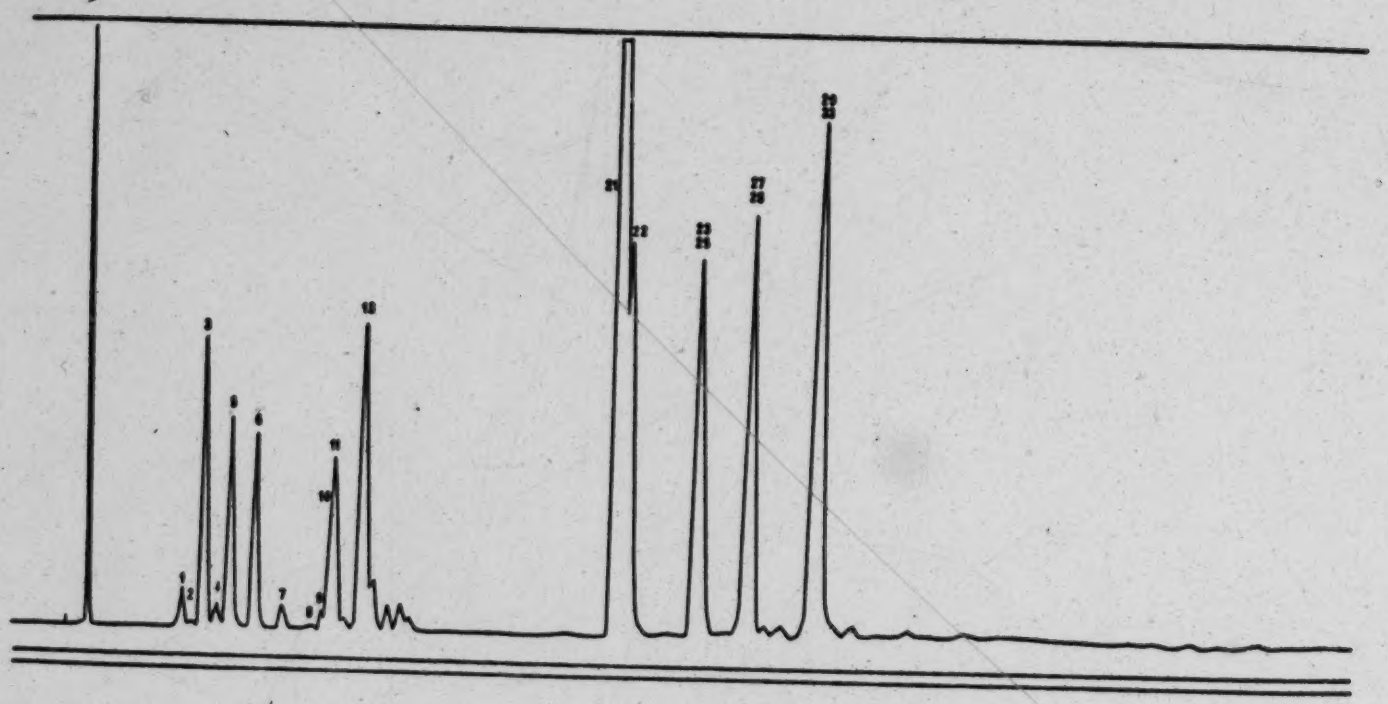


Fig. 28. Gas-Liquid Chromatogram of Sage Oil on the Fresh Column (Brooks--1971 Crop).

Peaks identified: (1)α-thujene, (3)α-pinene, (5) camphene, (6)β-pinene, (8)α-phellandrene, (10) myrcene, (11) limonene, (13) 1,8-cineol, (17) p-cymene, (19) fenchone, (21)α-thujone, (22)β-thujone, (23) camphor, (25) linalool, (27) bornyl acetate, (28)β-caryophyllene, (29) borneol, (33) humulene (α-caryophyllene).

sage oil from Brooks--1972 crop is given in Fig. 29. The peaks identified in the sage oil chromatograms were: α-thujene (1), α-pinene (3), camphene (5), β-pinene (6), α-phellandrene (8), myrcene (10), limonene (11), 1,8-cineol (13), p-cymene (17), fenchone (19), α-thujone (21), β-thujone (22), camphor (23), linalool (25), bornyl acetate (27), β-caryophyllene (28), borneol (29) and humulene (α-caryophyllene) (33). The GLC separation of the constituents of sage oil from Brooks--1971 crop showed five major peaks: 21 (α-thujone), 22 (β-thujone), 23 (camphor), 28 (β-caryophyllene) and 33 (humulene) while for the 1972 crop there were only four major peaks: 21 (α-thujone), 23 (camphor), 29 (β-caryophyllene) and 33 (humulene). The GLC separation of the constituents of sage oil from Michigan revealed four major peaks: 5 (camphene), 13 (1,8-cineol), 21 (α-thujone) and 23 (camphor). The medium peaks for the chromatogram of sage oil from Brooks--1971 crop were: 3 (α-pinene), 5 (camphene), 6 (β-pinene), 10 (myrcene), 11 (limonene), 13 (1,8-cineol), 27 (bornyl acetate) and 29 (borneol); while for the 1972 crop the medium peaks were: 3 (α-pinene), 5 (camphene), 6 (β-pinene), 11 (myrcene), 10 (limonene), 13 (1,8-cineol), 22 (β-thujone), 27 (bornyl acetate), 29 (borneol) and 54. The GLC separation of the constituents of sage oil from Michigan gave seven medium peaks: 3 (α-pinene), 11 (limonene), 22 (β-thujone), 27 (bornyl acetate), 28 (β-caryophyllene), 29 (borneol) and 33 (humulene).

The chromatogram of sage oil on the fresh column (see Fig. 27) showed that peak 10 (myrcene) was separated from peak 11 (limonene). However, peaks 21 (α-thujone) and 22 (β-thujone) were not resolved nor were 23 (camphor) and 25 (linalool), 27 (bornyl acetate) and 28 (β-caryophyllene), or 29 (borneol) and 33 (humulene). The GLC separation

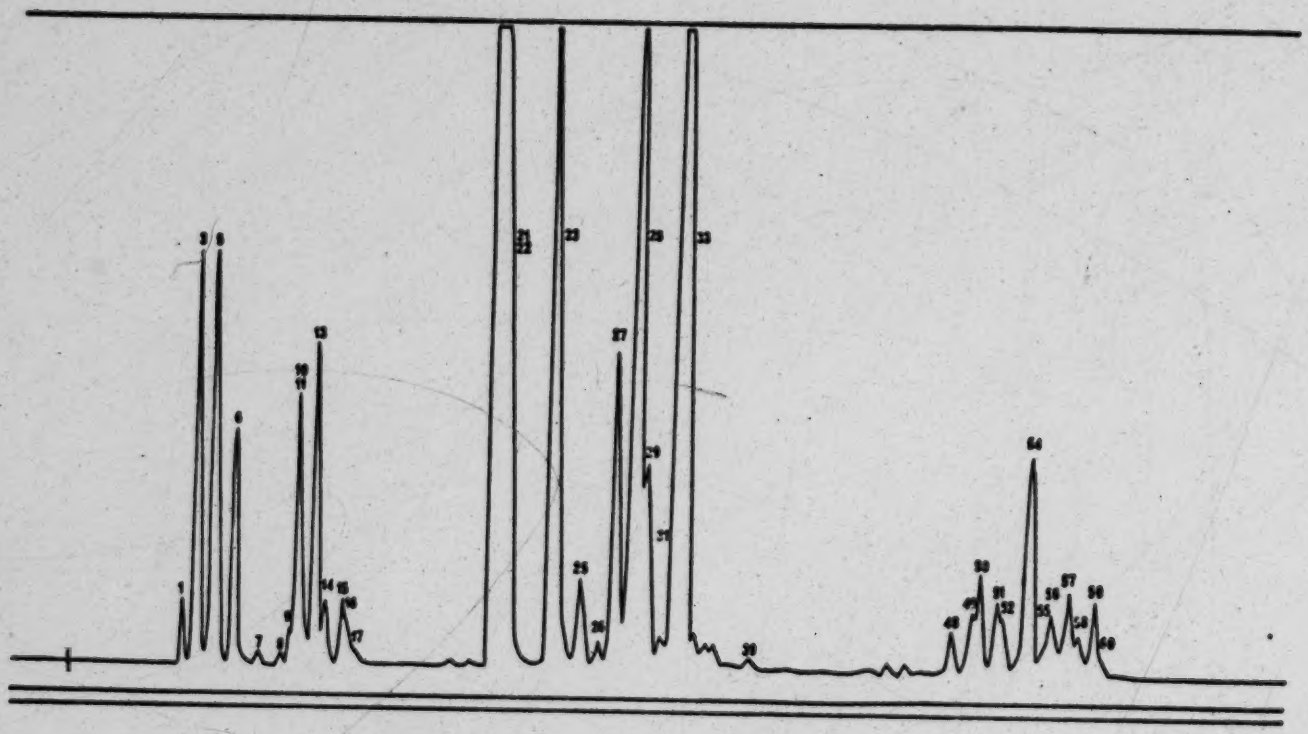


Fig. 29. Gas-Liquid Chromatogram of Sage Oil on the Aged Column (Brooks--1972 Crop).

Peaks identified: (1)α-thujene, (3)α-pinene, (5) camphene, (6)β-pinene, (8)α-phellandrene, (10) myrcene, (11) limonene, (13) 1,8-cineol, (17) p-cymene, (19) fenchone, (21)α-thujone, (22)β-thujone, (23) camphor, (25) linalool, (27) bornyl acetate, (28)β-caryophyllene, (29) borneol, (33) humulene (α-caryophyllene).

of sage oil on the fresh column (see Fig. 28) revealed that peak 1 (α-thujene) was separated from peak 2, and peak 3 (α-pinene) from peak 4. Peak 9 was partly separated from peak 10, as was peak 13 from 14, and peak 21 (α-thujone) from 22 (β-thujone). However, peak 9 (myrcene) was eluted almost as one peak with peak 11 (limonene). Camphor (23) and linalool (25) were eluted as one peak, as were bornyl acetate and β-caryophyllene (28), and borneol (29) and humulene (33). The chromatogram of sage oil in Fig. 29 shows that peak 1 (α-thujene) was eluted together with peak 2, peak 3 (α-pinene) with 4, peak 10 with 11, and peak 21 (α-thujone) with 22 (β-thujone). Peak 13 (1,8-cineol) was partly separated from peak 14 and peak 28 (β-caryophyllene) from 29 (borneol). Camphor (23) was separated from linalool (25), as was bornyl acetate (27) from β-caryophyllene (28) and borneol (29) from humulene (33).

3. Composition of Essential Oils

a. Anise Seed Oil

The percentage composition of anise seed oils from Brooks--1971 and 1972 crops and Kalamazoo, Michigan and the retention times of the identified constituents relative to trans-anethole, the major and most important constituent in anise oil, are shown in Table 22. The number of identified constituents was sixteen for Brooks--1971 crop, twelve for the 1972 crop and fifteen for Michigan, with the corresponding percentages of these constituents being 74.71, 84.56 and 90.48. The number of unidentified constituents for Brooks--1971 and 1972 crops and Michigan were twenty-three, eleven and nineteen, respectively, with corresponding percentages of 25.29, 15.50 and 9.72. The number of trace constituents was fifteen for Brooks--1971 crop, ten for the 1972 crop,

TABLE 22

THE COMPOSITION IN PERCENT OF ANISE SEED OIL

Constituent	Retention Time (trans-Anethole=1.00)*	Brooks, Alta. Crop 1971	1972	World Market Oil (Kalsec Int., Kalamazoo, Mich)
trans-anethole	1.00	57.42	75.21	72.23
cis-Anethole	0.91	1.80	1.06	2.46
Anisyl alcohol	1.23	0.24	0.45	0.92
Anisyl acetone	2.13	0.34	0.87	
p-Anisic acid	1.20	0.16	0.36	4.32
Camphor	0.69	0.28	0.15	0.20
Carvone	0.93	2.15	0.35	2.05
β-Caryophyllene	0.84	1.22	0.84	0.23
Dihydrocarvyl-acetate	0.91	1.43	0.88	0.41
Estragole	0.84	4.51	4.03	1.04
Eugenol	1.33	0.12	0.18	0.60
Fenchone	0.58	0.00	0.00	0.50
Limonene	0.34	3.96	0.00	4.90
Linalool	0.71	0.35	0.18	0.18
Myrcene	0.33	0.24	0.00	
α-Phellandrene	0.31	0.37	0.00	0.35
α-Pinene	0.19	0.12	0.00	0.09
TOTAL:				
Number of Identified Constituents		16	12	15
Corresponding %		74.71	84.56	90.48
Number of Constituents Unidentified		23	11	19
Corresponding %		25.29	15.50	9.72
Number of Constituents Present in Traces		15	10	7
Monoterpene hydrocarbons		6.24	0.30	6.39
Monoterpene alcohols		0.35	0.18	0.18
Monoterpene ketones		2.43	0.50	2.75
Sesquiterpene hydrocarbons		1.22	0.84	0.23
Benzene related constituents		64.59	82.16	81.57

*GLC separation data as on Fig. 15

and seven for Michigan.

In anise seed oil the percentages of monoterpene hydrocarbons were 6.24 for Brooks--1971 crop, 0.30 for the 1972 crop and 6.39 for Michigan. Only a small amount of monoterpene alcohols was found in anise oil: 0.35% for Brooks--1971 crop, 0.18% for the 1972 crop and 0.18% for Michigan. The highest amount of monoterpene ketones was in Michigan oil (2.75%), followed by Brooks--1971 oil (2.43%) and the 1972 oil (0.50%). The sesquiterpene hydrocarbons were present only in small amounts in anise oil: 1.22% for the Brooks--1971 crop, 0.84% for the 1972 crop and 0.23% for the Michigan oil. In contrast to the small amount of these terpene constituents anise oil contained a high amount of benzene related constituents. The most important and only major oil constituent is trans-anethole which amounted to 57.42% in Brooks--1971 crop, 75.21% in the 1972 crop and 72.23% in Michigan oil.

b. Caraway Seed Oil

The percentage composition of the caraway seed oils from Brooks--1971 crop and Kalamazoo, Michigan and retention times of the identified constituents relative to carvone, the most important constituent in caraway oil, are shown in Table 23. The number of identified constituents was eleven for Brooks oil and eight for Michigan oil, and the corresponding percentages of these constituents were 93.92 and 92.47. The number of unidentified minor consituents was twenty-three for Brooks oil and nine for Michigan oil and their corresponding percentages were 6.11 and 7.45 There were thirteen trace constituents for Brooks oil and twelve for Michigan oil.

TABLE 23

THE COMPOSITION IN PERCENT OF CARAWAY SEED OIL

Constituent	Retention Time (Carvone=1.00)*	Brooks, Alta. Crop	World Market Oil Michigan (Kalsec Int., Kalamazoo)
trans-Anethole	1.05	0.61	0.82
Camphor	0.72	0.09	0.0
β-Caryophyllene	0.88	1.17	1.67
Carvone	1.00	38.79	45.62
Limonene	0.37	48.77	43.17
Linalool	0.77	0.21	0.0
Myrcene	0.32	2.40	0.77
α-Phellandrene	0.33	0.28	0.08
α-Pinene	0.19	0.36	0.13
β-Pinene	0.26	0.31	0.10
α-Thujone	0.69	0.82	0.0
β-Thujone	0.70	0.18	0.11
Total:			
Number of Identified Constituents		12	9
Corresponding %		93.99	92.47
Number of Minor Constituents Unidentified		23	17
Corresponding %		6.11	7.45
Number of Constituents Present in Traces		13	12
Monoterpene hydrocarbons		54.17	45.51
Monoterpene alcohols		0.21	0.00
Monoterpene ketones ("carvone")		39.88	45.73
Sesquiterpene hydrocarbons		1.17	1.67
Benzene related constituents		0.61	0.82

*GLC separation data as on Fig. 19

In caraway oil the amount of monoterpene hydrocarbons was higher in oil from Brooks (54.17%) than that from Michigan (45.51%). There were no monoterpene alcohols identified in oil from Michigan, however there was 0.21% in oil from Brooks. The percentage of monoterpene ketones was higher in Michigan caraway oil (45.73%) than in Brooks oil (39.88%). The oil from Brooks had a smaller amount of sesquiterpene hydrocarbons (1.17%) than that from Michigan (1.67%). Unlike anise oil, caraway oil had only a small amount of benzene related constituents: 0.61% for Brooks and 0.82% for Michigan.

The two major and important constituents of caraway seed oil are carvone and limonene; their respective percentages were 38.79 and 48.77 for Brooks oil and 45.62 and 43.17 for Michigan oil.

c. Dill Oil

The percentage composition of dill seed oils from Brooks--1971 crop and dill herb oils from Kalamazoo, Michigan and the retention times of the identified constituents relative to carvone, one of the major and important constituents of dill oil, are given in Table 24. The three oils analyzed from Brooks were: dill sample A, dill sample B and Danish dill, and the two oils from Michigan were: dill standard and dill prime. There were ten identified constituents for dill oils from Brooks and nine for dill oils from Michigan. The percentages of these identified constituents were 94.29 for sample A, 94.26 for sample B, 90.14 for Danish dill, 89.27 for dill standard and 87.82 for dill prime. The number of unidentified constituents was fourteen for dill sample A, twelve for dill sample B, fifteen for Danish dill, sixteen for dill standard and fourteen for dill prime and the corresponding percentages of these

TABLE 24

THE COMPOSITION IN PERCENT OF DILL OIL

Constituent	Retention Time (Carvone=1.00)*	Brooks, Alta. A 1971	Brooks, Alta. B 1971	Danish	World Market Oil (Kalsec Int., Kalamazoo, Mich.) Standard	World Market Oil (Kalsec Int., Kalamazoo, Mich.) Prime
trans-Anethole	1.05	0.68	0.14	0.25	0.10	0.10
Camphene	0.20	0.07	0.11	0.13	0.21	0.29
Carvone	1.00	48.46	44.92	43.26	30.39	29.46
1,8-Cineol	0.39	1.07	1.42	1.11	0.57	6.87
p-Cymene	0.46	0.38	0.53	0.95	1.09	1.25
Limonene	0.37	38.27	40.82	33.11	37.62	26.04
Myrcene	0.35	0.74	0.93	1.21		
α-Phellandrene	0.34	4.30	4.96	9.66	18.03	22.50
α-Pinene	0.19	0.23	0.29	0.34	1.17	1.21
β-Pinene	0.26	0.09	0.14	0.12	0.09	0.10
Total:						
Number of Identified Constituents		10	10	10	9	9
Corresponding %		94.29	94.26	90.14	89.27	87.82
Number of Constituents Unidentified		14	12	15	16	14
Corresponding %		5.66	5.77	9.93	10.74	12.18
Number of Constituents Present in Traces		12	9	8	13	13

Table 24, Page 2 (continued) The Composition in Percent of Dill Oil

Constituent	Retention Time (Carvone=1.00)*	Brooks, Alta. A 1971	Brooks, Alta. B 1971	Danish	World Market Oil (Kalsec Int., Kalamazoo, Mich.) Standard	World Market Oil (Kalsec Int., Kalamazoo, Mich.) Prime
Monoterpene hydrocarbons		44.62	48.46	47.66	58.59	51.74
Monoterpene alcohols		0.68	0.68	3.42	5.86	6.87
Monoterpene ketones		48.46	44.92	43.26	30.39	29.46
Benzene related constituents		1.06	0.67	1.20	1.19	1.35

*GLC separation data as on Fig. 20

constituents were 5.66, 5.77, 9.93, 10.74 and 12.18. Dill sample A had twelve trace constituents, dill sample B nine, Danish dill eight, dill standard thirteen, and dill prime thirteen.

The dill oil contained a high percentage of monoterpene hydrocarbons: 44.62 in dill sample A, 48.46 in dill sample B, 47.66 in Danish dill, 58.59 in dill standard, and 51.74 in dill prime. The amounts of monoterpene alcohols in the dill oils were:0.68% dill sample A, 0.68% dill sample B, 3.42% Danish dill, 5.86% dill standard, and 6.87% dill prime. The only monoterpene ketone identified in dill oil was carvone. The amounts of benzene related constituents in dill oils were: 1.06% in dill sample A, 0.67% in dill sample B, 1.20% in Danish dill, 1.19% in dill standard and 1.35% in dill prime.

There are three important constituents in dill oil, namely carvone, limonene and α-phellandrene. The carvone content was 48.46% in sample A, 44.92% in sample B, 43.26% in Danish dill, 30.39% in dill standard and 29.46% in dill prime. The amount of limonene was 38.27% in sample A, 40.82% in sample B, 33.11% in Danish dill, 37.62% in dill standard and 26.04% in dill prime. Dill oil contained a higher percentage of α-phellandrene than other essential oils analyzed: 4.30 for sample A, 4.96 for sample B, 9.66 for Danish dill, 18.03 for dill standard and 22.50 for dill prime.

d. Fennel Seed Oil

The percentage composition of fennel oils from Brooks--1971 and 1972 crops, and from Fritzsche, and the retention times of the identified constituents relative to trans-anethole, the most important and a major constituentof fennel oil, are shown in Table 25. The number of

TABLE 25

THE COMPOSITION IN PERCENT OF FENNEL SEED OIL

Constituent	Retention Time (trans-Anethole=1.00)*	Brooks, Alta. Crop 1971	Brooks, Alta. Crop 1972	World Market Oil (Fritzsche,N.Y.)
cis-Anethole	0.93	0.43	0.58	0.43
trans-Anethole	1.00	39.19	68.90	57.15
Anisyl alcohol	1.21	0.0	0.0	0.71
p-Anisic acid	1.18	0.14	0.0	3.79
Borneol	0.84	0.12	0.0	2.42
Camphene	0.25	0.33	0.0	0.24
Camphor	0.68	3.55	0.60	4.31
Carvone	0.94	0.79	0.0	0.0
1,8-Cineol	0.38	1.12	0.69	0.0
p-Cymene	0.43	0.34	0.32	0.0
Estragole	0.85	4.08	6.09	4.18
Eugenol	1.29	0.18	0.0	0.0
Fenchone	0.63	16.12	10.83	4.31
Limonene	0.36	19.78	8.29	18.96
Myrcene	0.35	2.05	0.81	0.0
α-Phellandrene	0.33	0.43	0.18	0.0
α-Pinene	0.20	2.28	0.54	0.0
β-Pinene	0.27	0.45	0.25	0.0
α-Thujone	0.65	1.08	0.15	0.11
Total:				
Number of Identified Constituents		18	13	11
Corresponding %		92.46	98.23	96.61
Number of Minor Constituents Unidentified		20	5	9
Corresponding %		7.57	1.77	3.33
Number of Constituents Present in Traces		11	11	5
Monoterpene hydrocarbons		30.45	12.04	19.68
Monoterpene alcohols		0.12	0.00	2.42
Monoterpene ketones		20.81	11.58	4.31
Benzene related constituents		44.36	75.89	66.26

*GLC separation data as on Fig. 22

identified constituents was eighteen for Brooks--1971 crop, thirteen for the 1972 crop and eleven for Fritzsche oil, and the corresponding percentages were 92.46, 98.23 and 96.61. The number of unidentified minor constituents was twenty for Brooks--1972 crop, five for the 1972 crop and nine for Fritzsche with corresponding percentages of 7.57, 1.77 and 3.33. Both fennel oils from Brooks contained 11 trace constituents, while fennel oil from Fritzsche had only five trace constituents.

Fennel oil from Brooks--1971 crop had the highest amount of monoterpene hydrocarbons (30.45%) followed by fennel oil from Fritzsche (19.68%) and oil from Brooks--1972 crop (12.04%). The only monoterpene alcohol identified in this oil was borneol which amounted to 0.12% in oil from Brooks--1972 crop and 2.42% in oil from Fritzsche. The highest amount of monoterpene ketones was in fennel oil--1971 crop (20.81%), followed by oil--1972 crop (11.58%) and commercial oil from Fritzsche (4.31%). Fennel oil contained a high amount of benzene related constituents: 44.36% in fennel oil 1971, 75.89% in fennel oil 1972 and 66.26% in fennel oil, Fritzsche.

The important constituents of fennel seed oil are trans-anethole and fenchone. Their corresponding percentages were 39.19 and 16.12 for Brooks--1971 crop, 68.90 and 10.83 for 1972 crop, and 57.15 and 4.31 for fennel oil from Fritzsche, N.Y. The other major constituent in fennel oil is limonene which amounted to 19.78% in Brooks--1971 crop, 8.29% in the 1972 crop and 18.96% in the oil marketed by Fritzsche.

e. Peppermint Oil

The percentage composition of the leaf peppermint oils from Brooks--1970 crop, Beaverlodge--1973 crop and Michigan (Hotchkiss, Lyons,

N.Y.) is given in Table 26. The peppermint oil samples from Brooks were obtained at five stages of growth: stage I (bud stage-harvested August 1), stage II (beginning of blooming-harvested August 8), stage III (75% blooming-harvested August 22), stage IV (full bloom-harvested August 29) and stage V (end of blooming-harvested September 12). The peppermint oils from Beaverlodge were designated sample A and sample B. The table also includes the retention times of the identified constituents relative to menthol, which is the major and the most important constituent of peppermint oil.

The number of identified constituents was sixteen in the Beaverlodge peppermint oils and twenty-one in the other oils. The percentage of identified constituents was: for Brooks, 93.64 in stage I, 92.89 in stage II, 93.61 in stage III, 91.82 in stage IV and 93.05 in stage V; for Beaverlodge, 99.08 in sample A and 96.02 in sample B; and 92.34 for Michigan oil. The unidentified minor constituents were: fourteen for stage I, thirteen for stage II, twenty-one for stage III, thirteen for stage IV, fifteen for stage V, four for sample A, nine for sample B and thirteen for Michigan oil, and their respective percentages were: 7.60, 7.91, 7.05, 7.91, 6.71, 2.14, 8.40 and 7.67. The number of the trace constituents was twenty in stage I, twenty-one in stage II, thirteen in stage III, twenty-one in stage IV, eighteen in stage V, twelve in sample A, eight in sample B and nineteen in Michigan oil.

Peppermint oil had a low amount of monoterpene hydrocarbons: 8.76% in stage I, 7.60% in stage II, 7.02% in stage III, 7.77% in stage IV, 8.07% in stage V, 0.15% in sample A, 0.0% in sample B and 6.79% in Michigan oil. Of the six essential oils analyzed, peppermint oil con-

TABLE 26

THE COMPOSITION IN PERCENT OF PEPPERMINT OIL

Constituent	Retention Time (Menthol=1.00)*	Brooks, Alta. I**	II	III	IV	V	World Market Oil Michigan (Hotchkiss, Lyons, N.Y.)	Beaverlodge, Alta. A	B
Camphene	0.14	0.34	0.32	0.25	0.32	0.31	0.17	0.0	0.0
β-Caryophyllene	0.93	3.00	1.80	1.65	1.77	1.67	2.26	0.76	0.0
Cineol (1,8)	0.34	5.10	5.24	5.70	5.70	5.13	4.19	3.96	4.24
p-Cymene	0.41	0.47	0.45	0.50	0.35	0.33	0.38	0.19	0.0
Isomenthol	1.05	0.63	0.86	0.50	0.88	0.60	0.69	0.66	0.36
Isomenthone	0.84	3.49	3.02	3.38	2.86	2.80	3.33	3.31	4.40
Limonene	0.29	1.61	1.63	1.53	1.72	2.03	1.57	0.15	0.0
Linalool	0.90	0.58	0.50	0.35	0.66	0.49	0.55	0.71	0.93
Menthofuran	0.74	1.52	1.07	0.90	1.73	2.89	2.00	0.54	0.28
Menthol	1.00	32.33	36.63	40.33	41.23	44.18	42.76	53.83	58.94
Menthone	0.77	32.62	29.52	26.02	22.93	21.24	19.35	26.24	11.85
Menthyl acetate	0.95	2.07	2.39	2.69	2.79	2.83	5.56	0.73	1.89
Myrcene	0.27	0.88	0.63	0.69	0.98	0.71	0.73	0.0	0.0
Neoisomenthol	0.95	0.11	0.32	0.75	0.10	0.46	1.22	0.0	0.0
Neomenthol	0.91	3.49	2.97	3.38	3.44	3.28	4.19	5.77	3.12
3-Octanol	0.59	0.56	0.62	0.70	0.45	0.61	0.43	0.51	1.97
α-Phellandrene	0.26	0.39	0.39	0.30	0.41	0.30	0.20	0.0	0.0
α-Pinene	0.12	0.71	0.66	0.55	0.57	0.60	0.48	0.0	0.0
β-Pinene	0.19	1.04	0.95	0.75	0.85	0.93	0.81	0.0	0.0
Piperitone	1.29	1.00	1.11	1.24	0.95	0.85	0.62	1.56	6.58
Pulegone	1.11	0.84	1.12	0.80	1.10	0.88	0.85	0.43	1.96
Total: Number of Identified Constituents		21	21	21	21	21	21	15	12

Table 26, Page 2 (continued) The Composition in Percent of Peppermint Oil

Constituent	Retention Time (Menthol=1.00)*	Brooks, Alta. I**	II	III	IV	V	World Market Oil Michigan (Hotchkiss, Lyons, N.Y.)	Beaverlodge Alta. A	B
Corresponding %		92.78	92.10	93.06	91.82	93.05	92.34	99.08	96.02
Number of Constituents Unidentified		14	13	21	13	15	13	4	9
Corresponding %		7.60	7.91	7.05	7.91	6.71	7.67	0.93	4.02
Number of Constituents Present in Traces		20	21	13	21	18	19	12	8
Monoterpene hydrocarbons		8.79	7.60	7.02	7.77	8.07	6.79	0.15	0.0
Monoterpene alcohols		42.24	46.52	51.01	52.10	54.14	53.10	60.26	62.42
Monoterpene ketones		37.95	34.77	31.44	27.84	25.77	24.15	31.54	24.79
Esterified "Menthol"		2.07	2.39	2.69	2.79	2.83	5.56	0.73	1.39
Sesquiterpene hydrocarbons		3.00	1.80	1.65	1.77	1.67	2.26	0.76	0.0
Benzene related constituents		0.47	0.45	0.50	0.38	0.33	0.38	0.19	0.0

*Conditions of separation as on Fig. 24
**Harvest time: I, Bud Stage (Aug. 1); II, Beginning of Blooming (Aug. 8); III, Blooming 75% (Aug. 22); IV, Full Bloom (Aug. 29); and V, End of Blooming (Sept. 12)

tained the highest amount of monoterpene alcohols. The amounts of these monoterpene alcohols were: 42.24% in stage I, 46.52% in stage II, 51.01% in stage III, 52.10% in stage IV, 54.14% in stage V, 60.26% in sample A, 62.42% in sample B and 53.10% in Michigan oil. The amounts of monoterpene ketones were: 37.95% in stage I, 34.77% in stage II, 31.44% in stage III, 27.84% in stage IV, 25.77% in stage V, 31.54% in sample A, 24.79% in sample B and 24.15% in Michigan oil. The only sesquiterpene hydrocarbon found in peppermint oil was β-caryophyllene which amounted to 3.00% in stage I, 1.80% in stage II, 1.65% in stage III, 1.77% in stage IV, 1.67% in stage V, 0.76% in sample A, 0.00% in sample B and 2.26% in Michigan oil.

The two major and important constituents of peppermint oil are menthol and menthone. In Brooks peppermint leaf oils menthol content increased from 32.62% at the bud stage to 44.18% at the end of flowering. The amounts of menthol in Beaverlodge oils A and B and Michigan oil were: 53.83%, 58.94% and 42.76%, respectively. The menthone content in Brooks peppermint leaf oil decreased from 32.62% at the bud stage to 21.24% at the end of flowering. The amounts of menthone in Beaverlodge oils A and B and Michigan oil were: 26.24%, 11.85% and 19.35%, respectively.

The esterified menthol, menthyl acetate, and menthofuran are two additional important constituents in peppermint oil. The amounts of menthyl acetate and menthofuran were, respectively, 2.07% and 1.52% in stage I, 2.39% and 1.07% in stage II, 2.69% and 0.90% in stage III, 2.79% and 1.73% in stage IV and 2.83% and 2.89% in stage V, 1.03% and 0.54% in sample A, 1.29% and 0.49% in sample B, and 5.56% and 2.00% in Michigan oil. There was only a small amount of benzene related consti-

tuents present in peppermint oil: 0.47% in stage I, 0.45% in stage II, 0.50% in stage III, 0.38% in stage IV, 0.33% in stage V, 0.99% in sample A, 0.00% in sample B and 0.38% in Michigan oil.

The percentage composition of the peppermint stem oils from Brooks is given in Table 27. The samples were obtained from three stages of growth: stage I (bud stage-harvest August 1), stage III (75% blooming-harvested August 22) and stage V (end of blooming-September 12). The number of identified constituents was twenty-one for both stage I and stage III and nineteen for stage V, and their respective percentages were: 93.46, 92.67 and 94.44. The unidentified minor constituents were: thirteen for stage I, twenty-one for stage III and eleven for stage V, with corresponding percentages of 6.71, 7.24 and 5.55, respectively. Stage I contained 6.17% monoterpene hydrocarbons, stage III 5.36% and stage V 6.40%. The peppermint stem oil had a higher amount of monoterpene alcohols than the leaf oil: 48.64% in stage I, 44.32% in stage III, and 69.67% in stage V. On the other hand, the amount of monoterpene ketones was lower in the stem oil than in the leaf oil: 27.77% in stage I, 32.67% in stage III and 13.01% in stage V. The amounts of sesquiterpene hydrocarbons were: 2.59% in stage I, 5.33% in stage III and 2.16% in stage V. The menthol content in stem oil was: 41.80% in stage I, 39.90% in stage III and 63.95% in stage V. The amounts of menthone were 22.92% in stage I, 27.25% in stage III and 9.66% in stage V. The percentage of menthyl acetate was higher in the stem than the leaf: 2.63% in stage I, 3.03% in stage III and 3.31% in stage V. The menthofuran content was 2.04% in stage I, 1.28% in stage III and 0.60% in stage V. The amount of benzene related constituents in stem oil was: 0.32% in stage I, 0.50% in stage III and 0.58% in stage V.

TABLE 27

THE COMPOSITION IN PERCENT OF PEPPERMINT STEM OIL

Constituents	Brooks, Alta. I	III	V
Camphene	0.27	0.15	0.26
β-Caryophyllene	2.59	1.71	1.18
1,8-Cineol	5.63	5.33	2.16
p-Cymene	0.32	0.50	0.58
Isomenthol	1.94	0.81	1.20
Isomenthone	2.99	3.71	1.71
Limonene	0.98	1.21	0.64
Linalool	0.74	0.31	0.50
Menthofuran	2.04	1.28	0.60
Menthol	41.80	39.90	63.95
Menthone	22.92	27.25	9.66
Menthyl acetate	2.63	3.03	3.31
Myrcene	0.78	0.22	0.78
Neoisomenthol	0.26	0.43	1.25
Neomenthol	3.90	2.87	2.77
3-Octanol	0.65	1.10	0.85
α-Phellandrene	0.32	0.17	0.57
α-Pinene	0.27	0.37	0.37
β-Pinene	0.57	0.61	0.46
Piperitone	0.94	1.22	0.96
Pulegone	0.92	0.49	0.68
Total:			
Number of Identified Constituents	21	21	21
Corresponding %	93.46	92.67	94.44
Number of Minor Constituents Unidentified	13	21	11
Corresponding %	6.71	7.24	5.55
Monoterpene hydrocarbons	6.17	5.36	6.40
Monoterpene alcohols	48.64	44.32	69.67
Monoterpene ketones	27.77	32.67	13.01
Sesquiterpene hydrocarbons	2.59	5.33	2.16
Esterified "Menthol"	2.63	3.03	3.31
Menthofuran	2.04	1.28	0.60
Benzene related constituents	0.32	0.50	0.58

f. Sage Oil

The percentage composition of sage oils from Brooks--1971 and 1972 crops and from Kalamazoo, Michigan and the retention times of the identified constituents relative to camphor, which is one of the major constituents in sage oil, are shown in Table 28. The number of identified constituents in these three sage oils was 20 and the percentages of these identified constituents were 86.48 for Brooks--1971 crop, 82.96 for the 1972 crop and 94.20 for Michigan sage oil. The number of unidentified minor constituents and the corresponding percentage were twenty-seven and 13.15% for 1971 sage; twenty-eight and 15.83% for 1972 sage; and fifteen and 6.36% for Michigan sage oil. There were 13 trace constituents in both Brooks sage oils but no trace constituents in Michigan oil.

Sage oil--1971 crop contained 21.77% monoterpene hydrocarbons, sage oil--1972 crop 15.02% and Michigan sage oil 20.23%. The amount of monoterpene alcohols was 8.95% in sage oil--1971 crop, 9.67% in sage oil--1972 crop and 15.23% in Michigan oil. The percentage of monoterpene ketones was 37.51 in sage oil--1971 crop, 34.08% in sage oil--1972 crop and 49.50 in Michigan sage oil. The amount of monoterpene esters was 2.70% in sage oil--1971 crop, 3.69% in sage oil--1972 crop and 2.97% in Michigan sage oil. Of the six essential oils analyzed, sage oil contained the highest amount of sesquiterpene hydrocarbons: 20.79% in sage oil--1971 crop, 25.78% in sage oil--1972 crop, and 10.03% in Michigan oil. Like peppermint, caraway and dill oils, sage oil contained small amounts of benzene related constituents: 0.16% in sage oil--1971 crop, 0.12% in sage oil--1972 crop and 0.16% in Michigan oil.

TABLE 28

THE COMPOSITION IN PERCENT OF SAGE OIL

Constituent	Retention Time (Camphor=1.00)*	Brooks, Alta. Crop 1971	1972	World Market Oil (Kalsec Int., Kalamazoo, Mich.)
Borneol	1.20	1.57	2.41	4.29
Bornyl acetate	1.13	2.33	3.19	2.63
Camphene	0.29	3.77	3.79	7.24
Camphor	1.00	8.34	10.31	25.60
β-Caryophyllene	1.17	7.67	9.34	4.55
Cineol (1,8)	0.50	5.69	3.43	10.00
p-Cymene	0.59	0.16	0.12	0.16
Estragole	1.29	0.34	0.43	0.43
Fenchone	0.79	0.11	0.07	0.23
Humulene	1.27	13.12	16.44	5.48
Limonene	0.47	2.99	1.25	2.53
Linalool	1.05	0.68	1.15	0.54
Myrcene	0.47	1.58	1.07	1.11
α-Phellandrene	0.44	0.19	0.11	0.13
α-Pinene	0.26	4.56	3.57	3.41
β-Pinene	0.34	3.68	2.05	1.36
α-Thujene	0.23	0.60	0.53	0.84
α-Thujone	0.89	21.53	20.47	19.95
β-Thujone	0.92	7.53	3.23	3.72
Total:				
Number of Identified Constituents		19	19	19
Corresponding %		86.48	82.96	94.20
Number of Minor Constituents Unidentified		27	28	15
Corresponding %		13.57	17.23	6.07
Number of Constituents Present in Traces		13	13	0
Monoterpene hydrocarbons		21.77	15.02	20.23
Monoterpene alcohols		8.95	9.67	15.23
Monoterpene ketones		37.51	34.08	49.50
Monoterpene esters		2.33	3.19	2.63
Sesquiterpene hydrocarbons		20.79	25.78	10.03
Benzene related constituents		0.16	0.12	0.16

*GLC separation data as on Fig. 29

The most important constituent in sage oil is thujone (α- and β-), which amounted to 29.06% in sage oil from Brooks--1971 crop, 23.70% from the 1972 crop oil and 23.67% from Michigan oil.

E. Biochemical Changes in Composition During the Growth Period of Peppermint Herb

The percentages of the four most important constituents of peppermint oil from Brooks in relation to the stages of growth, together with the biochemical relationships of the major monoterpenes in mint plant, as outlined by Waller (1970), are shown in Fig. 30. The four constituents plotted in this figure are menthol, menthone, menthyl acetate and menthofuran. Menthol and menthone are plotted from data for both leaf and stem oils, whereas data on menthyl acetate and menthofuran are only from the leaf oils. The amount of menthol, both in the stem and the leaf, increases as the plant grows. In the leaf, the amount of menthone decreases steadily as the plant matures but in the stem in increases slightly at stage III (75% blooming) and decreases drastically at the end of flowering. Menthyl acetate content of the leaf increases steadily as the plant matures. This coincides with the increase of menthol in the leaf. These data are in agreement with the biochemical relationship between menthone, menthol and menthyl acetate as proposed by Waller (1970). As the plant grows menthone is reduced to menthol which is then esterified slowly to menthyl acetate. In the case of menthofuran, its content decreases up to stage III (75% blooming) and then increases as the plant produces more flowers.

The percentage composition of neomenthol, isomenthone, piperitone and pulegone in the stem and the leaf of peppermint plant from

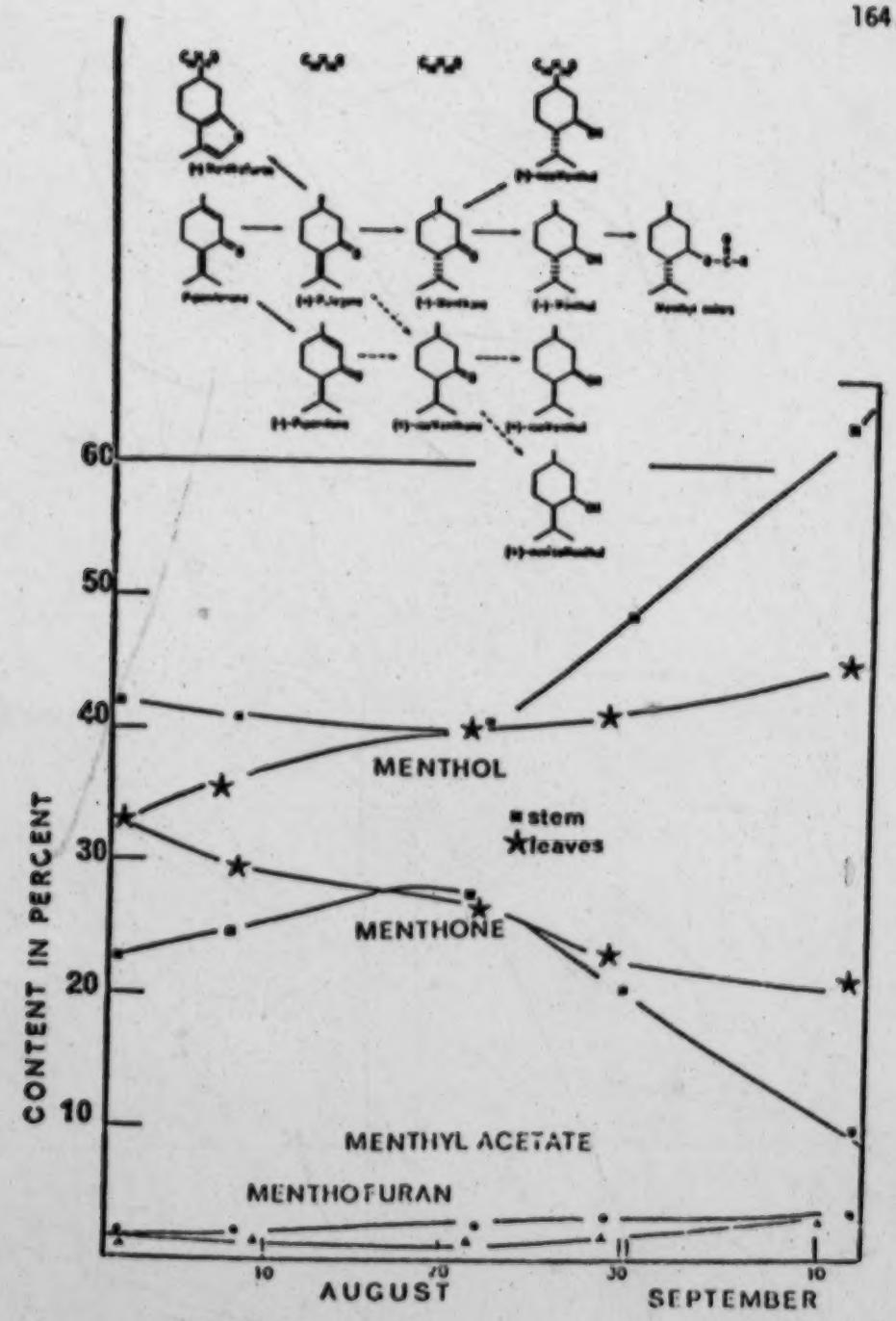


Fig. 30. Biochemical Changes in Composition During the Growth Period of Peppermint Herb.

Brooks in relation to the stages of growth, together with biochemical relationships of the major monoterpenes in mint plant, according to Waller (1970), are given in Fig. 31. The neomenthol content of the stem decreases with plant maturity, however, in the leaf it decreases slightly up to the stage II and then increases with the plant growth. The isomenthone content of the stem increases up to stage III and then decreases as the plant matures. In the leaf, the isomenthone content decreases up to stage II (beginning of blooming), increases to stage III (75% blooming) and then decreases again until the end of flowering. The amount of piperitone in both the stem and leaf increases slightly with plant growth. The pulegone content of the leaf increases up to stage III (75% blooming) and then decreases as the plant matures, while the opposite is true in the stem where it decreases up to stage III and then increases until the end of flowering.

F. Significant Constituent Ratios for Peppermint Oil

The significant constituent ratios for peppermint oil (according to Smith and Levi, 1961) from Brooks, Beaverlodge and Michigan, are given in Table 29. The ratio of monoterpene hydrocarbons plus 1,8-cineol to the total amount of constituents is designated as ratio A. The value of ratio A in the leaf peppermint oil from Brooks ranged from 0.109 at the beginning of blooming to 0.121 at full bloom. The stem peppermint oil had comparatively lower values of ratio A which decreased from 0.102 at the bud stage to 0.066 at the end of blooming. The value of ratio A for Beaverlodge samples A and B, and Michigan oils was 0.040, 0.43 and 0.091, respectively.

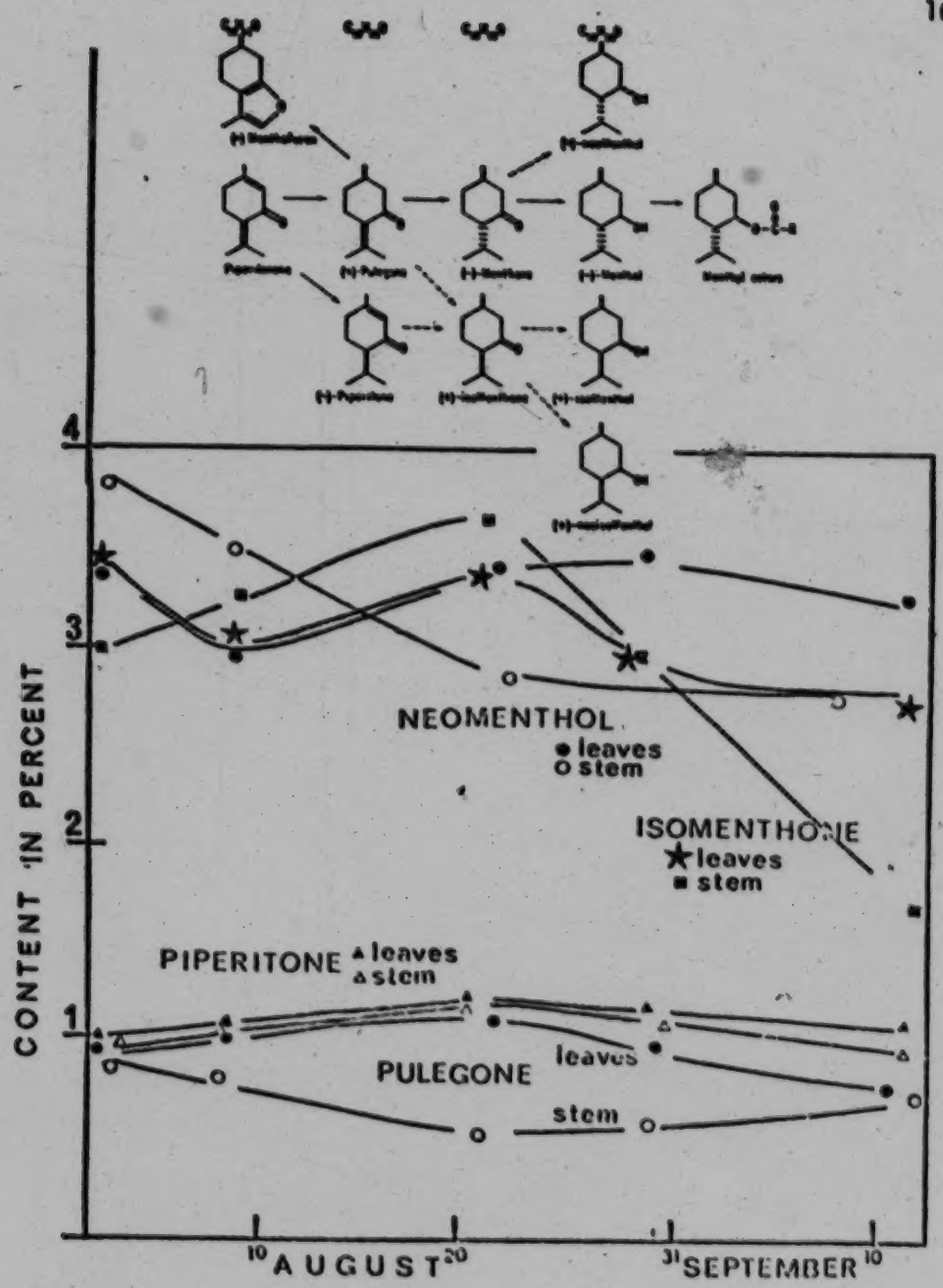


Fig. 31. Biochemical Changes in Composition During the Growth Period of Peppermint Herb.

TABLE 29

SIGNIFICANT CONSTITUENT RATIOS FOR PEPPERMINT OILS

Sample	Percentage Ratio A: Terpenes* / All Constituents	Percentage Ratio B: Menthone / Isomenthone	Percentage Ratio C: Limonene / 1,8-Cineol
BROOKS, ALTA.			
Leaf			
Bud Stage	0.116	9.35	0.32
Beginning of Blooming	0.109	9.77	0.31
Blooming 75%	0.116	7.70	0.27
Full Bloom	0.121	8.02	0.30
End of Blooming	0.115	7.59	0.40
Stem			
Bud Stage	0.102	7.67	0.17
Blooming 75%	0.090	7.27	0.23
End of Blooming	0.066	5.65	0.21
BEAVERLODGE, ALTA.			
Sample A	0.040	7.93	0.04
Sample B	0.043	2.69	0.00
MICHIGAN (Hotchkiss, Lyons, N.Y.)	0.091	5.81	0.37

Table 29, Page 2 (continued)

Significant Constituent Ratios For Peppermint Oils

Percentage Ratio			
D	E	F	G
Menthofuran / "Menthone Related Constituents+"	Neomenthol / Menthyl Acetate	"Menthol Related Constituents+" / Neomenthol	"Menthone Related Constituents" / "Menthol Related Constituents"
0.040	1.69	11.07	0.97
0.018	1.24	14.53	0.78
0.029	1.26	14.09	0.64
0.063	1.23	14.08	0.57
0.107	1.16	15.65	0.52
0.074	1.48	12.95	0.55
0.040	0.95	16.39	0.69
0.050	0.84	26.09	0.17
0.02	7.90	10.57	0.49
0.02	2.24	20.45	0.26
0.081	0.75	12.99	0.45

*"Terpenes": principal constituents emerging prior to and including 1,8-cineol (α-pinene, camphene, β-pinene, limonene and 1,8-cineol)
†"Menthone related constituents": menthofuran, menthone and isomenthone
+"Menthol related constituents": neomenthol, menthol, menthyl acetate, isomenthol and neoisomenthol

The ratio of menthone to isomenthone is designated as ratio B. The leaf peppermint oil, with relatively higher values of ratio B than the stem oil, had values ranging from 7.59 at the end of blooming to 9.77 at the beginning of blooming. The ratio B values for the stem peppermint oil decreased from 7.67 at the bud stage to 5.65 at the end of blooming. The values of ratio B for Beaverlodge, samples A and B, and for Michigan oil were: 7.93, 2.69 and 5.81, respectively.

The ratio of limonene to 1,8-cineol is designated as ratio C. In the leaf oil, the C:ratio values were between 0.27 at 75% blooming and 0.40 at the end of blooming, while the stem oil values were lower than those of the leaf oil and ranged between 0.17 at the bud stage to 0.23 at 75% blooming. The ratio C for Michigan oil was 0.37. The oils from Beaverlodge had low values of ratio C; 0.04 for sample A and zero for sample B.

The ratio of menthofuran to "menthone related constituents", which include menthofuran, menthone and isomenthone, is designated as ratio D. The value of ratio D in the leaf oil increased from 0.018 at the beginning of blooming to 0.107 at the end of blooming, while in the stem oil the values ranged from 0.040 at 75% blooming to 0.074 at the bud stage. The D:ratio values for Michigan oil and for both oil samples from Beaverlodge were 0.081 and 0.02, respectively.

The ratio of neomenthol to menthyl acetate is designated ratio E. In the leaf oil this value decreased from 1.69 at the bud stage to 1.16 at the end of blooming, while in the stem oil it decreased from 1.48 at the bud stage to 0.84 at the end of blooming. The Michigan oil had the lowest E ratio value at 0.75, while the Beaverlodge oils had the highest E ratio values, 7.90 in sample A and 2.24 in sample B.

The ratio of "menthol related constituents" to neomenthol is designated as ratio F. The "menthol related constituents" include menthol, menthyl acetate, neomenthol, isomenthol and neoisomenthol. The F ratio value in the leaf oil ranged from 11.07 at the bud stage to 15.65 at the end of blooming, while in the stem oil it increased from 12.95 at the bud stage to 26.09 at the end of blooming. The Michigan oil F value of 12.99 was relatively low when compared to those of Brooks oils. The values of ratio F for Beaverlodge samples A and B were 10.57 and 20.45, respectively.

The ratio of "menthone related constituents" to "menthol related constituents" is designated as ratio G. Its value in the leaf oil decreased from 0.97 at the bud stage to 0.52 at the end of blooming, while in stem oil the values ranged from 0.17 at the end of blooming to 0.69 at 75% blooming. The G value of 0.45 for Michigan oil was lower than that of Brooks oils. The G values for Beaverlodge oils were 0.49 for sample A and 0.26 for sample B.

By plotting certain significant ratios, it is possible to distinguish peppermint oils by their geographical origin (Smith and Levi, 1961). The plot of ratio F (menthol related constituents/neomenthol) against ratio G (menthone related constituents/menthol related constituents) for peppermint oil is illustrated in Fig. 32. The geographical regions shown are U.S.-Yakima, Oregon and Michigan, England, Italy (Smith and Levi, 1961) and Brooks, Alta., with data for Brooks and Yakima from both early and late harvests. It is not representative to plot the data for Beaverlodge since only two samples were analyzed. The plot can be used to differentiate the geographical origin of the

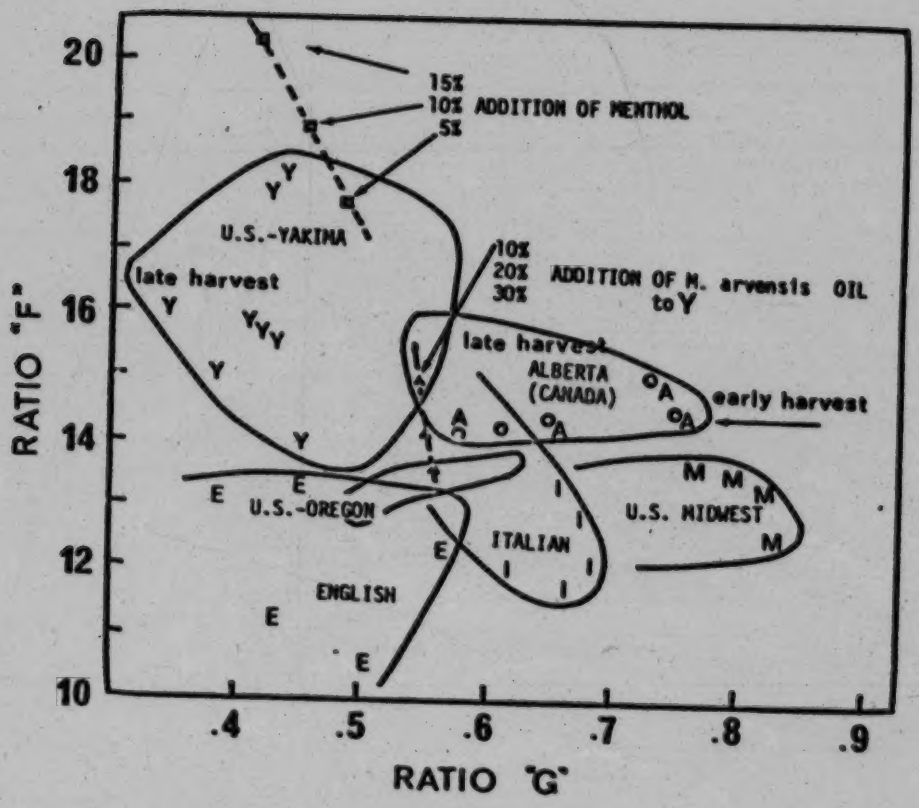


Fig. 32. Distribution Plot of Ratio F ("menthol related constituents"/ neomenthol) vs Ratio G ("menthone related constituents"/"menthol related constituents").

peppermint and to reveal the adulteration pattern and the oil due to the addition of menthol or oil of Mentha arvensis L.

The distribution plot of ratio D (menthofuran/menthone related constituents) against ratio E (neomenthol/menthyl acetate) for peppermint oil is shown in Fig. 33. This plot can also be used to differentiate the oils by geographical origins and to show the pattern of adulteration with oil Mentha arvensis L. The geographical regions shown are U.S.-Yakima, Oregon and Michigan, Italy based on data obtained by Smith and Levi (1961) and Brooks, Alta., with data for Brooks and Yakima for both early and late harvests.

The distribution plot of ratio C (limonene/1,8-cineol) against ratio D (menthofuran/menthone related constituents) is illustrated in Fig. 34. The plot is used to distinguish the mint oils either from M. piperita L. or from M. arvensis L. This plot confirmed that mint oils from Brooks and Beaverlodge are from M. piperita L.

G. The Quality of Essential Oils

1. Anise Seed Oil

The quality of anise oil depends mainly on the amount of trans-anethole (Guenther, 1950; Anonymous, 1970a). Arctander (1969) stated that trans-anethole is the compound which imparts a "very sweet, herbaceous warm odour and a sweet taste" to the oil. The amount of this compound in essential oils of anise seed has been reported to be high and to range between 80 and 90% (Guenther, 1950). The Egyptian anise oil was reported by El-Deeb et al. (1962a) to contain 88.61% anethole. Tsvetkov (1970) in a study of Bulgarian anise seed oil observed that the anethole content of the central, first order, and second order um-

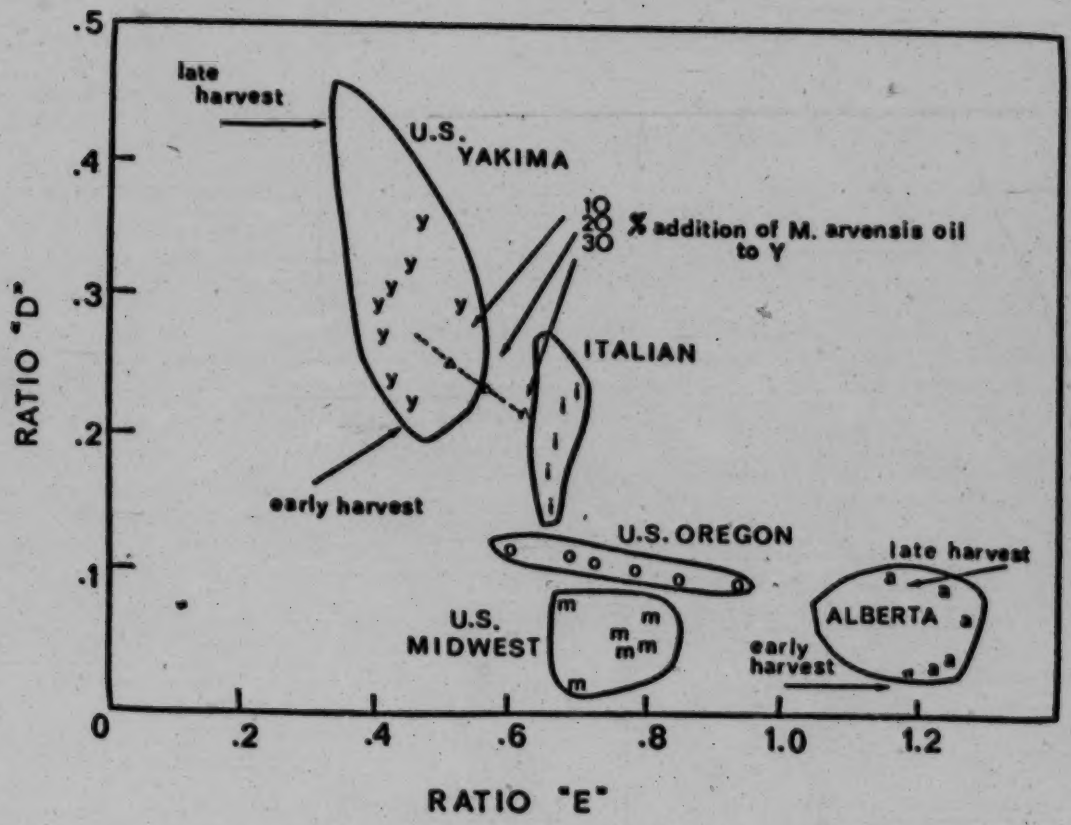


Fig. 33. Distribution Plot of Ratio D (menthofuran/"menthone related constituents") vs Ratio E (neomenthol/menthyl acetate).

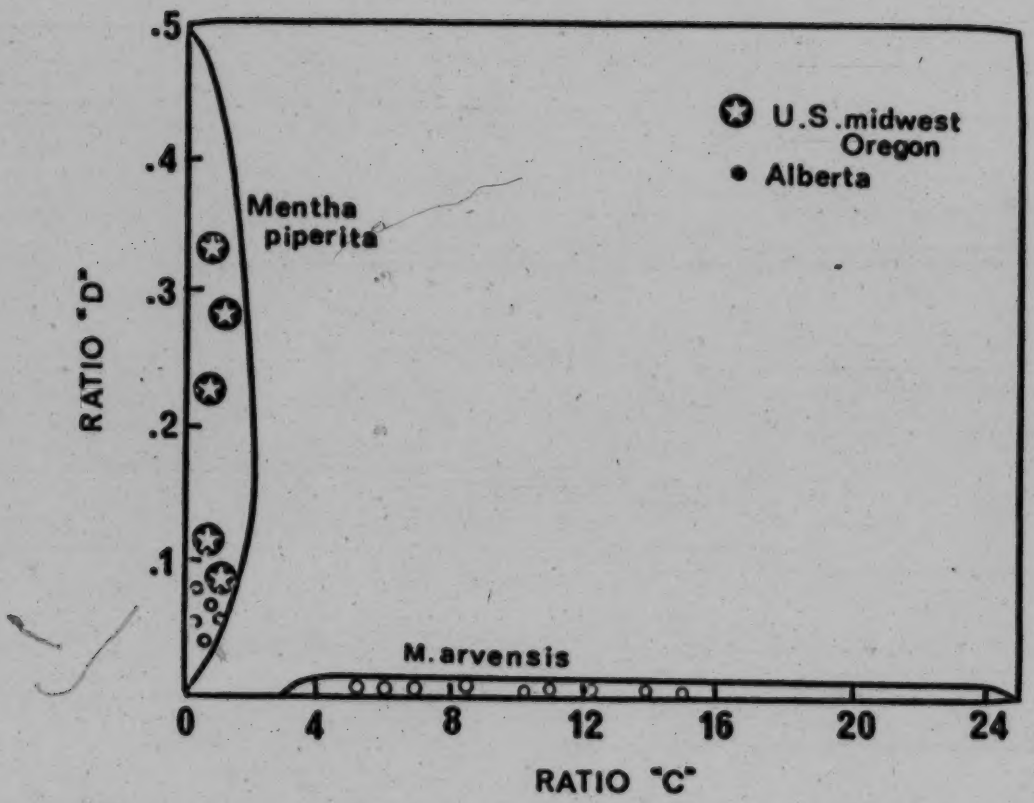


Fig. 34. Distribution Plot of Ratio C (limonene/1,8-cineol) vs Ratio D (menthofuran/"menthone related constituents")

bels was 87.30%, 89.50% and 88.40%, respectively, and concluded that a high quality anise oil could be obtained when the fruits in central umbels were ripe. In this study it was found that the trans-anethole content of anise oil from Brooks--1971 crop and 1972 crop, and from Michigan was 57.4%, 75.2% and 72.2%, respectively. Hence, anise oils from Brooks and Michigan were of lower quality than those reported in the literature. Of interest is the fact that the quality of anise oils for the 1971 crop was lower and for the 1972 crop higher than that of anise oil from Michigan.

Cis-anethole is considered 15 times more toxic than trans-anethole and its consumption can be hazardous (Anonymous, 1970a). The amounts of cis-anethole in anise oil from Brooks--1971 and 1972 crops, and Michigan were 1.8%, 1.1% and 2.5%, respectively. In respect to its low cis-anethole content anise oil from Brooks, especially the 1972 crop, is superior to Michigan oil.

Methyl chavicol (estragole), an isomer of anethole, has a "sweet herbaceous anise-fennel type odor, much more herbaceous than anethole but not the sweet flavor of anethole" (Arctander, 1969). Tsvetkov (1970) reported that the estragole content of the central, first order and second order umbels was 8.64%, 7.51% and 7.71%, respectively. In this study it was found that anise oil from Brooks contained a higher amount of estragole (4.5% in the 1971 crop, 4.0% in the 1972 crop) than the Michigan oil (1.0%). Hence, this component adds to the quality of Brooks oil.

Anisyl acetone has an "intensely sweet, floral and slightly fruity, cherry-preserve-like odor of good tenacity" (Arctander, 1969).

The results obtained in this study showed that Michigan anise oil did not contain anisyl acetone while anise oil from Brooks--1972 crop had a higher amount of anisyl acetone (0.9) than the 1971 crop (0.3).

When all these constituents are taken into consideration it is obvious that anise oil from Brooks--1972 crop was of higher quality than the oils from Michigan and Brooks--1971 crop. The difference in composition was significant, especially in the amount of trans-anethole. The difference in quality of the oils from Brooks was due to the fact that the oil obtained from the 1971 crop was distilled from the whole plant whereas the oil obtained from the 1972 crop was distilled only from the seed.

The yield of anise seed oil from Brooks was reported only for the 1971 crop as 1.05%. According to Gildermeister and Hoffmann (1931), the yield of anise seed oil of various origins ranged from 1.5 to 6.0%. Fisher et al. (1945) reported that the yield of commercial anise seed oil from various origins varied from 1.9 to 3.1% with an average of 2.29%. Therefore, the yield of Brooks anise seed oil has to be considered as low.

2. Caraway Seed Oil

The most important constituent and the main carrier of the characteristic odor of caraway oil is D-carvone (Anonymous, 1970b; Guenther, 1950). It has a "warm herbaceous, breadlike, spicy and slightly floral odor and a warm, sweet, spicy-herbaceous, breadlike taste" (Arctander, 1969). A good quality caraway oil should contain 50 to 60% carvone (Guenther, 1950; Anonymous, 1970b). Caraway oil from Egypt contained 56.9% carvone (El-deeb et al., 1962a); from Holland and Russia

51.0 to 59.0%, and from Ohio 59.7% (Fritzche Brothers Inc. Labs., 1950). Caraway oil from Brooks--1971 crop contained only 38.8% carvone, while the oil from Michigan contained 45.6%. The results showed that the quality of caraway oil from Brooks is lower than that from Michigan and other countries. However, a low carvone content in caraway oil does not indicate inherent carvone deficiency in the fruit (Betts, 1965). Other factors such as the weather (Guenther, 1950) and the state of maturity of the plant (Sandermann, 1938) and seed (Schimmel & Co., 1896) also determine the amount of carvone in the oil. The carvone content is highest in fully matured seed (Schimmel & Co., 1896). Sunny dry weather during seed maturation causes an increase in carvone content, whereas cool damp weather reduces the amount of carvone and other oxygenated compounds. The caraway plant from Brooks--1971 crop in general experienced a cool growing season from May to mid-July. The weather was hot and sunny from mid-July to the beginning of September. The caraway oil from Brooks--1971 crop was distilled from the harvest which had only about 50% of mature seeds. Thus, the amount of carvone in caraway oil from Brooks can be increased by harvesting the plant when all the seeds are mature.

The other important constituent of caraway oil is limonene. It has a "fresh, light and sweet citrusy odor with a strong resemblance to orange peel oil but with poor tenacity. Its taste is sweet, refreshing, citrus-like, orange-like and mild." (Arctander, 1969). The amount of limonene in caraway oil depends indirectly on the content of carvone and other oxygenated components. El-Deeb et al. (1962a) obtained 45.6% limonene; Ikeda et al. (1962) 38.0%; and Sandermann (1938) 75.0% from flowering caraway plants. In this study it was found that the oil from

Brooks had a higher limonene content (48.8%) than that from Michigan (43.2%).

The yield of caraway seed oil from Brooks was recorded only for 1971 crop as 2.65%. This yield was lower than that of Dutch caraway seed oil which varied from 3 to 6% with an average of 4%, however, it is higher than the range of 0.4 to 2% reported for North Africa and Near East oils (Guenther, 1950).

3. Dill Oil

There are two types of dill oil, dill herb and dill seed oil. The quality of dill herb oil depends on the amount of α-phellandrene and carvone (Guenther, 1950) and possibly on the amount of limonene, the major constituent. α-Phellandrene has a "pleasant, freshly citrusy and peppery-woody odour with a discretely minty note but poor tenacity." (Arctander, 1969). The higher the amount of this constituent the better the quality of the oil and the more the odor and smell of the oil resembles that of the fresh herb. The fresh herb character can be detected if the amount of carvone is less than 35%. The oils containing 20% or less carvone have the finest and most pronounced herb character (Guenther, 1950). In this study it was found that both Michigan oils, dill prime and standard, were herb oils because they had a lower carvone content and a higher content of α-phellandrene. Dill standard oil contained 30.4% carvone, and dill prime oil had 29.5%. The limonene and α-phellandrene contents were 37.6% and 18.0%, respectively, for dill standard oil, and 26.0% and 22.5% for dill prime oil. Thus dill prime oil is considered to be a better quality oil than dill standard oil.

The dill oils from Brooks were distilled from dill seed. The dill seed oil contained a high amount of carvone and, hence, has an odor and flavor similar to that of caraway oil. The results showed that the major difference between caraway and dill oils was the amount of α-phellandrene, which was 4.3% and 5.0% in common dill oils and 9.7% in Danish dill oils but only 0.1 to 0.3% in caraway oils.

The quality of dill seed oil depends mainly on the amount of carvone; the higher the carvone content, the better the quality. The quality also depends on two other major constituents, limonene and α-phellandrene. The amount of carvone was 48.5% in common dill sample A, 44.9% in common dill sample B and 43.2% in Danish dill. The carvone content of dill seed oils from India was 41.8 to 62.2%, Bulgaria 39.0%, Ireland 37.8% (Virmani and Datta, 1970a), America 50.0 to 60.0%, Hungary 40.0 to 68.0%, Russia 53.6% and England 48.0 to 57.0 (Guenther, 1950). Thus, the quality of dill seed oil from Brooks--1971 crop was comparable to that of oil from other parts of the world. The carvone content of dill herb oil depends on the state of ripeness of the herb material (Guenther, 1950; Luyendijk, 1957); the more mature the plant, the higher the amount of carvone. The dill seed oil from Brooks was distilled from the harvest with 50% matured seeds. The amount of carvone in the dill seed oil from Brooks can be increased by distilling the seeds when they are fully mature.

The amount of α-phellandrene in Danish dill oil was twice as much as that of the common dill oils. Since α-phellandrene can bring out the fresh herb character, Danish dill oil was better in this respect. The limonene content of the oil was highest in common dill sample B, followed by common dill sample A and Danish dill.

The oil yields of Danish dill seed and common dill seed from Brooks--1971 crop were 1.18% and 1.82%, respectively. These yields from Brooks were lower than those from other countries which ranged from 2.1% in India to 5.62% in Russia.

4. Fennel Seed Oil

The quality of fennel seed oil varies with different varieties; sweet fennel seed oil is of highest quality because it contains no or a low amount of fenchone and the highest amount of anethole; bitter fennel seed oil is of lower quality oil since it contains a high amount of fenchone and a slightly lower amount of anethole; wild bitter fennel is of lowest quality because it contains a very low amount of anethole (Guenther, 1950). The results of this study revealed that the fennel plant from Brooks is actually bitter fennel. The quality of bitter fennel seed oil depends on the amounts of trans-anethole and fenchone. Trans-anethole has a "very sweet, herbaceous warm odor and a sweet taste" (Arctander, 1969). The higher the amount of trans-anethole, the better the quality of the oil. A good quality of bitter fennel seed oil contains 50.0 to 60.0% of trans-anethole (Guenther, 1950). The anethole content of bitter fennel seed oil (by congealing point) from Austin, Minnesota is 65.0% and from the U.S.S.R. 61.0 to 62.5%. In this study it was found that the trans-anethole content of the bitter fennel oil from Brooks--1971 crop was 39.2%; Brooks--1972 crop, 68.9%; and the commercial oil from Fritzsche, 57.2%. The amount of trans-anethole from Brooks--1972 crop was higher than that of oils from Austin, the U.S.S.R. and Fritzsche. Thus, the quality of bitter fennel seed oil from the Brooks--1971 crop was poorer and from the 1972 crop was better than that

of Fritzsche.

Another factor which determines the quality of bitter fennel seed oil is the amount of fenchone. D-fenchone has an "intensely bitter, camphor-like odor and flavor and is responsible for somewhat coarse bitter, taste" (Guenther, 1950). Arctander (1969) described the odor of fenchone as "warm-camphoraceous, powerful, diffusive, and basically sweet and its taste as warm, somewhat burning and bitter". The lower the amount of fenchone, the better the quality of bitter fennel oil. The amount of fenchone, which varies in oils obtained from different hybrids of bitter fennel seed, ranged from 16.3 to 31.6 parts relative to 100 parts of trans-anethole (Karlsen et al., 1969). The results of this study showed that bitter fennel seed oil from the Brooks--1971 crop had the highest amount of fenchone (16.1%), followed by the 1972 crop (10.8%), and Fritzsche (4.3%). Karlsen et al. (1969) observed that the green fruits of fennel contain relatively more fenchone than brownish ones. There was no information given on the state of maturity of either of the crops of fennel seed harvested at Brooks.

The difference in quality between the fennel oils from Brooks--1971 and 1972 crops was due to the fact that the volatile oil from the 1971 crop was distilled from the whole plant whereas volatile oil from the 1972 crop was distilled only from the seeds. Thus, a good quality of fennel oil from Brooks is obtainable by distilling the mature seeds.

The yield of fennel seed oil from the Brooks--1971 crop was 1.44%. According to Guenther (1950), the oil yield of the best grade of fennel seed of eastern Europe was between 2.5 and 5.0%. The oil yield of sweet and bitter fennel seeds varied between 1.50 and 3.82%

and 2.92 and 5.76%, respectively (Karlsen et al., 1969). Nigerian sweet fennel seed was reported to yield 2.0 to 2.4% oil (Osisiogu, 1967). Thus, the yield of fennel seed oil from Brooks was lower than that of the other regions.

5. Peppermint Oil

The characteristic peppermint oil flavor is apparently due to the combined effect of several constituents in the oil (Pintauro, 1971). There are four stereoisomers of menthol in peppermint oil: menthol, neomenthol, isomenthol and neoisomenthol. Of the four stereoisomers, only menthol (the main constituent of the peppermint oil) is present in a high amount. Neomenthol is one of the intermediate constituents of peppermint oil. Neoisomenthol and isomenthol are only present in small amounts. Arctander (1969) described the odor of L-menthol as "refreshing, light, diffusive, sweetly pungent with resemblance to the main odor of peppermint, cooling mouthfeel, and taste that is refreshing, sweet and reminiscent of part of peppermint picture". It has a cooling effect upon mucous membranes. D-neomenthol has a refreshing menthol type odor but is not as sweet as menthol. It has a taste similar to that of menthol at a very low concentration, but at a higher concentration the cooling effect is less than that of L-menthol. It carries with it a musty or flat odor that tends to detract from the peppermint aroma rather than lifting it. Of the four stereoisomers, L-menthol is responsible for the agreeable odor of peppermint, while neomenthol, isomenthol and neoisomenthol exhibit increasingly musty odors (Hornstein and Teranishi, 1967). Our study has shown that the menthol content of the leaf and stem oils increased with plant maturity. This agreed with previous literature

data. Menthol content of the leaf peppermint oil from Brooks increased from 32.3% at the bud stage to 44.2% at the end of blooming. The menthol content of oil from Michigan was 42.8% from Beaverlodge sample A 53.8% and sample B 58.9%. The peppermint oil distilled from the stem had a relatively high menthol content; 39.9% at 75% blooming and 63.9% at the end of blooming. The quality of peppermint oil depends mainly on the amount of menthol; the higher the menthol content the better the quality (Virmani and Datta, 1970b; Guenther, 1952). Hence, the quality of the oil from Brooks increased with the maturity of the plant. The menthol content in peppermint oils from Beaverlodge was higher than that from Brooks and Michigan. The menthol content of peppermint oil from Brooks was higher than that of Michigan oil when the oil was distilled from the later harvests.

The amount of neomenthol in peppermint leaf oil from Brooks ranged from 3.0% at the beginning of blooming to 3.5% at the bud stage and was lower than that from Michigan (4.2%). For Beaverlodge peppermint oils, sample A had a higher neomenthol content (5.8%) than sample B (3.1%). The neomenthol content of the stem oil from Brooks decreased from 3.9% at the bud stage to 2.8% at the end of blooming. An excessive amount of neomenthol lowers the quality of the oil. The effect of neomenthol on the aroma and flavor could be detected significantly at a level of 7.5% (Cash et al., 1971).

Menthyl acetate is another important constituent which affects the quality of the oil. L-menthyl acetate has a "mild, sweet, slightly fruity-herbaceous, minty odor and a quite refreshing and delicately floral, cool mouthfeel, sweet taste with only a trace of mintness. Its

overall flavor is much milder than L-menthol but more delicate and floral" (Arctander, 1969). The higher the amount of menthyl acetate, the better the quality of the oil (Virmani and Datta, 1970b). The results of our study showed that the amount of menthyl acetate in leaf peppermint oils from Brooks increased with the maturity of the plant: 2.1% at the bud stage to 2.8% at the end of blooming. The oil from the stem contained a higher amount of menthyl acetate, ranging from 2.6% at the bud stage to 3.3% at the end of blooming. The Beaverlodge peppermint oils contained a very low amount of menthyl acetate: 0.7% in sample A and 1.4% in sample B. The oil from Michigan contained the highest amount of menthyl acetate (5.6%).

Menthone is also a characteristic and valuable component of peppermint oil (Pintauro, 1971). Menthone has a "minty, refreshing and diffusive odor of moderate tenacity and slightly woody-dry undertone and a refreshingly cool, but also unpleasantly bitter taste in dilutions of 10 to 50 ppm. Isomenthone has a powerful, refreshing, clean-minty odor of moderate tenacity and somewhat cooling mouthfeel and refreshing, minty but distinctly bitter taste" (Arctander, 1969). Due to its bitter taste an excessive amount of menthone is undesirable (Guenther, 1952). An excessive amount of isomenthone, which has a bitter taste, is also undesirable. This study has shown that the amounts of menthone and isomenthone in the leaf oils from Brooks decreased with plant maturation. This agreed with previous findings. The lowest amount of menthone was found in Beaverlodge peppermint oil sample B (11.9%). The amount of menthone in Michigan oil (19.3%) was lower than that in Brooks oils. The menthone content of the leaf peppermint oils from Brooks decreased from 32.6% at the bud stage to 21.2% at the end of blooming. In the stem

peppermint oils menthone content ranged from 27.2% at 75% blooming to 9.7% at the end of bloom. The highest amount of isomenthone was found in Beaverlodge sample B (4.4%). Michigan oil and Beaverlodge oil sample B contained the same amount of isomenthone. In Brooks peppermint leaf oils, the isomenthone content decreased from 3.5% at the bud stage to 2.8% at the end of blooming. In stem oils, the amount of isomenthone ranged from 3.7% at 75% blooming to 1.7% at the end of blooming.

The quality of the peppermint oil from Brooks improved as the plants matured due to the increase in the amounts of menthol and menthyl acetate and the decrease in the amounts of menthone and isomenthone. However, there were two negative factors, namely the low oil yield and the menthofuran content, which offset this trend. The percentage yield of oil increases until the plant reaches the full blooming stage and then decreases rapidly as the foliage begins to fall (Guenther, 1952).

Watson and John (1955) were the first to report the association of a poor peppermint oil quality with a high menthofuran content. Menthofuran has a "sweet, hay-like-minty odor, sometimes referred to as a 'lactone' odor of moderate to poor tenacity and a slightly bitter, almost tarry and unpleasant taste. Its initial impression is that of a very pungent and sharp odor" (Arctander, 1969). The menthofuran present in freshly distilled oil is not too objectionable insofar as its effect on the quality of the oil, but it can absorb oxygen from the air to form compounds, which may possibly include peroxides, that decompose or react with other constituents of the oil resulting in discoloration and in a bitter taste or "after flavor" (Pintauro, 1971). Wood and Eastman (1950) have shown that menthofuran readily undergoes autoxidation to form a

lactone. The effect of menthofuran on the flavor of peppermint oil can be detected significantly at levels of 9.5% or higher (Cash et al., 1971). The amount of this compound was first associated with the number of blossoms at harvest time (Watson and John, 1955). Later, Lemli (1957) established that menthofuran is a substance secreted in the young parts of the plant, where metabolism is most active. Thus, menthofuran content is highest in the young plants and decreases as the plant grows, but increases again from the time of flower bud formation and finally, decreases after blooming. In this study it has been shown that the menthofuran content in the peppermint leaf oils from Brooks decreased from 1.5% at the bud stage to 0.9% at 75% blooming and then increased to 2.9% at the end of blooming. Michigan peppermint oil contained 2.0% of menthofuran, while that from Beaverlodge contained a very low amount: 0.5% in sample A and 0.3% in sample B. The amount of menthofuran in peppermint stem oil from Brooks decreased from 2.0% at the bud stage to 0.6% at the end of blooming.

The peppermint stem oil is of better quality than the leaf oil because it contains comparatively higher amounts of menthol and menthyl acetate and lower amounts of menthone, isomenthone, menthofuran and neomenthol. However, the stem usually yields low amounts of oil (Guenther, 1952), hence, its contribution to the quality of the peppermint oil can not be significant.

Michigan peppermint oil is considered as one of the finest quality peppermint oils available on the market. The results of this study showed that the quality of Brooks peppermint oil at the late harvest was comparable to that of Michigan oil. The most significant difference between oils from Michigan and Brooks at the late harvest stage was the

amount of menthyl acetate and neomenthol. The low amount of neomenthol in Brooks peppermint oil is a desirable characteristic of a good quality oil while the low menthyl acetate content in Brooks oil is an undesirable characteristic.

The quality of peppermint oil from Beaverlodge sample B was better than that of sample A because sample B oil contained lower amounts of menthone and neomenthol and higher amounts of menthol and menthyl acetate. The quality of Beaverlodge sample B oil was quite comparable to that of Michigan oil. However, Beaverlodge oils contained a very low amount of menthyl acetate, in fact, lower than that of Brooks oil.

According to the biochemical relationships of menthone related constituents given by Waller (1970) free menthol is esterified to menthyl acetate. The low amount of menthyl acetate in Alberta oils could be due to the cooler climate of Alberta, the shorter period of sunshine and the shorter frost free period in the growing season of only four to five months in Brooks. Beaverlodge, which is in Northern Alberta, has a comparatively cooler climate, a lesser amount of sunshine and a shorter frost free period in the growing season than Brooks. Hence, the amount of menthyl acetate in the peppermint oil is lower than that from Brooks.

The menthofuran content of peppermint oil from Alberta peppermint oils was low when compared with other oils. Loomis (1967) has shown that high night temperatures and more profuse blooming cause a high menthofuran content. Since the night temperatures in Alberta are normally low, the amount of menthofuran in Alberta peppermint oil is also low. Similarly, due to the lower night temperatures at Beaverlodge,

the amount of menthofuran is even lower than that of Brooks.

The yield of peppermint herb oil from Brooks increased from 0.20% at the bud stage to 0.22% at the beginning of blooming and then decreased to 0.18% at the end of blooming. The oil yield from Brooks peppermint herb was lower than that of American and European peppermint herbs. According to Mikhalov (1929), the oil content in the leaves is a direct function of the mean temperature of the growing season. The mean temperatures of the growing season at Brooks were 59.6° for 1971 and 60.2° for 1972. The mean temperature of the growing season at Beaverlodge was expected to be lower. Thus, due to the low mean temperature of the growing season, the yield of peppermint herb oil in Alberta is expected to be lower than yields in the U.S. and Europe.

6. Sage Oil

Guenther (1952) described the odor and flavor of genuine Dalmation sage oil as being strongly aromatic and somewhat spicy. Thujone is the most important constituent of sage oil and is largely responsible for its characteristic odor and flavor, thus a high percentage of thujone indicates a good quality. As stated by Arctander (1969), thujone has a "powerful, penetrating, warm-herbaceous, and minty-camphoraeous odor of moderate to poor tenacity and a warm-herbaceous bitter taste in concentrations lower than 10 ppm and a pungent-bitter herbaceous taste at higher concentrations". In the literature the amount of thujone is usually reported as part of the ketone content which also includes other ketones such as camphor and fenchone. The amount of thujone in Dalmation sage was reported to be 51.0% (Brickorn and Wenger, 1960).

In this study the amounts of both α- and β-thujone were reported. Of the three oils analyzed, sage oil from Brooks--1971 crop contained the highest amount of thujone (α-thujone, 21.53%; β-thujone, 7.53%). The thujone content of sage oil from Brooks--1972 crop and Michigan was about the same: α-thujone 20.5% and β-thujone 3.2% (Brooks--1972 crop) and α-thujone 19.9% and β-thujone 3.7% (Michigan oil). Thus, the sage oil from Brooks--1971 crop was of better quality than that from Brooks--1972 crop and Michigan. However, the quality of these three sage oils was lower than that of the original Dalmation sage.

The other major constituent of sage oil is humulene (α-caryophyllene) which amounted to 13.1% in Brooks--1971 crop oil, 16.4% in the 1972 crop and 5.48% in Michigan oil. The amounts of β-caryophyllene were 7.7% in sage oil from Brooks--1971 crop, 9.3% in the 1972 crop and 4.6% in sage oil from Michigan. Both α- and β-caryophyllene have a "woody-spicy, dry and tenacious odor and a dry woody, somewhat bitter taste" (Arctander, 1969). The presence of humulene and β-caryophyllene in such a high percentage, especially in sage oils from Brooks, definitely enhances the odor and flavor of the sage oil.

Camphor is also a major constituent in sage oil, especially in Michigan oil (25.6%). The amounts of camphor in sage oils from Brooks were relatively low: 8.3% in the 1971 crop and 10.3% in the 1972 crop. Camphor has a "warm-minty almost ethereal-diffusive odor of low tenacity and a slightly bitter, warm taste" (Arctander, 1969). Camphor, in such a high percentage,especially in Michigan sage oil, definitely influences the quality of the oil.

1,8-cineol has a "fresh, diffusive, camphoraceous-cool odor of poor tenacity and a sweet, fresh cool camphoraceous taste" (Arctander,

1969). The amount of 1,8-cineol was 5.7% in sage oil from Brooks--1971 crop, 3.4% in the 1972 crop; and 10.0% in Michigan sage oil. 1,8-cineol, especially the high content in Michigan oil, adds to the camphoraceous odor and taste of sage oil. Thus, Michigan sage oil had a more camphoraceous odor and taste than sage oils from Brooks.

As shown earlier, there is a difference in the composition and the quality of sage oils from Brooks between the 1971 crop and the 1972 crop. The sage oil from 1971 was of better quality than that from the crop year 1972. The oil for the 1971 crop was obtained from distilling the whole, dried plant harvested at 70% blooming. There is no data available from Brooks about the stage of plant maturity at the harvest time for the crop year 1972. Weather data showed that the number of hours of sunshine and the number of frost free days were greater for the 1971 growing season than for the 1972 growing season with the reverse being true with respect to the amount of rainfall. If the stage of the plant at the harvest time for the 1971 and 1972 crops was the same, the better quality oil from the 1971 crop could be due to the longer period of sunshine and the greater number of frost free days.

The yield of sage oil from Brooks for the 1971 crop was reported as 0.25%. There was no data on the oil yield for the 1972 crop. This yield of oil was very much lower than that reported by Guenther (1952) for Dalmation sage (0.7 to 2.0%) and for American sage (0.6 to 1.0%). The yield of oil could be increased by harvesting at an earlier stage of maturity.

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APPENDIX A: Agrological Requirements for Growing the Spices and Herbs

A-1

ANISE

Growing Season	Climate	Soil	Yield/Acre
1. Growing seazon should be frost free at least for 120 days. 2. Vegetative periods are 130 to 140 days. With short growing season it is difficult for the plant to mature. 3. a. In USA the seedlings are adversely affected by transplanting. The seeds are sown directly in the field in early spring at 5 to 10 lb of seed/acre - 2.5 cm in the row of 46 to 76 cm apart. When the seedlings are 5 to 7.5 cm high they are thinned to 15 cm apart in the row. b. In Europe the seed is broadcast.	1. The plant needs a cool, wet climate. 2. It requires uniform rainfall during growing season. 3. It is susceptible to extreme climatic changes from wet to dry periods. 4. Alternate rainy and dry periods cause the seed to turn brown, thus greatly impairing the quality. 5. It needs a fairly uniform temperature throughout the growing season, without excessive hot periods particularly after rainfall. 6. It needs a warm and sunny weather condition but extremely hot and dry weather affects the maturation of crop.	1. The plant needs a light, well drained, fertile or moderatlly rich sandy loam. 2. The soil should be well pulverized and able to retain moisture.	400-800 lb of seed

A-2

CARAWAY

Growing Season	Climate	Soil	Yield/Acre
1. Caraway is a biennial 2. Dutch caraway needs 15 months. 3. American caraway needs a shorter growing season of approximately 11 to 14 months. Seed matures even at short growing season. 4. a. Dutch caraway seeds are planted in rows of 30 to 46 cm apart, at 5 to 8 lb/acre. b. American caraway seeds are sown in rows about 46 cm apart at 6 to 8 lb/acre.	1. Dutch caraway grows well in temperature and humid climate especially during winter. It is susceptible to frost damage. 2. American caraway withstands quite a severe continental climate. It is adapted to cool climate of temperate region but not suited to warm climate. 3. Rainfall or wind might result in complete loss of the crop.	1. Dutch caraway requires a fertile soil. 2. It needs somewhat clayey soil containing a fair proportion of humus. 3. It needs a loamy or stiff clay soil. 4. The plant grows well in any good upland soil.	800-12,000 lb of seed

DILL

Growing Season	Climate	Soil	Yield/Acre
1. In USA and Europe: from very early spring to late summer (about 165 days). 2. Under climate of India: a. Monson crop(herb)--134 days b. Winter crop(herb)--162 days c. Winter crop(seed)--212 days 3. a. In USA seeds are sown at 1.5 cm deep, at 36 to 46 cm in rows of 30 to 92 cm apart. The amount of seeds required is 7 lb/acre. At proper time the plants are thinned to 15 to 38 cm apart. b. In Europe seeds are sown usually at 30 cm apart; when the soil is free of weed--at 11 to 13 cm apart; when planting for seed--at 35 to 40 cm apart; the amount of seeds required is 12 to 16 lb/acre.	1. The plant needs a cooler climate with warm dry intervals. 2. It is more susceptible to weather hazards. Hail, strong winds and driving rains may injure the flowering plants resulting in no yield of oil.	1. The plant is most suited to well drained, fertile, sandy loam soil. 2. It grows well in any good garden. Light sandy or heavy clayey soil is not suitable for growing this plant. 3. The soil should be deeply ploughed and harrowed. The soil must be smooth and free of clods.	500-700 lb of seeds which give after steam distillation 20 to 30 lb of oil.

A-4

FENNEL

Growing Season	Climate	Soil	Yield/Acre
1. Fennel is a herbaceous perennial.	1. It needs sunny localities with a fairly mild climate.	1. Plant grows in any good soil.	600-1,400 lb of seed
2. It is grown from early spring to September next year--about 16 to 17 months of growing season.	2. Too much mositure favors excessive development of leaves and stalks rather than seeds.	2. It thrives best in rich well-drained loam or black, sandy and sandy clay, soils containing sufficient lime.	
3. Plant can be grown from seed and propagated by root and crown divisions. Seeds are grown in rows of 61 to 92 cm apart at 7 to 9 lb/acre. The established plants are thinned in the row to 30 cm apart.			

PEPPERMINT

Growing Season	Climate	Soil	Yield/Acre
1. Peppermint is a perennial. 2. Growing period is 5 to 7 months depending on weather. 3. It is propagated by stolons. In USA-- 10 to 15 cm in the row of 91 cm apart. In Europe-- 10 to 30 cm. Transplanting reduces the crop loss.	1. a. Oil content is a direct function of the mean temperature during the growth. b. 20°C is the optimum for leaf development, lateral branching and initiation and development of flowers. c. Frost and cold weather retard growth. 2. a. A short day light (10 to 13 h) results in no bud formation, with oil containing very small amount of menthone and menthol and high amount of menthofuran--up to 84%. b. Long day light (14 to 18 h) results in bud formation and flowering, greater number of oil gland/unit area of leaves lower epidermis, with high yield oil containing low amount of menthone and menthofuran and high amount of menthol (56%). c. Prolonged spells of cloudy or rainy weather	1. a. For optimum growth and oil yield, the plant needs nonacid soil of pH 6.0 to 7.5 with pH 5.0 to 8.0 as a wider limit. b. For higher menthol and ester contents, a light, sandy or loamy rather than heavier soil is needed. c. The plant thrives best in a deep rich, well-drained open texture soil. 2. After ploughing, the field is disced and thoroughly harrowed. Loose soil should be rolled to firm it. 3. a. NPK fertilization effects include:	USA 30 to 92 lb of oil England 20 to 40 lb France 54 to 107 lb

Peppermint

Growing Season	Climate	Soil	Yield/Acre
	result in low menthol and high menthone contents.	increase in oil yield and herb and increase in quality especially less fertile soils. b. In reclaimed peat bog, N increases the oil yield but reduces the menthol content; superphosphate reduces only menthol while a mixture of NPK reduces the oil yield. c. For highly organic muck soils, the recommended treatment is 300 to 500 lb/acre of 5-20-20 fertilizer; for cool and wet seasons 25 to 50 lb of N/acre as side dress is needed. d. On established planting, the recommended treatment is 250 to 500 lb/acre of 5-20-20 fertilizer. e. For sandy loam soils the recommended treat-	

Growing Season	Climate	Soil	Yield/Acre
		ment is 120 lb N/ acre. 4. a. The plant requires considerable amount of water throughout growing season. Lack or excess of water reduces the oil content. b. In summer the plant needs 127 to 152 cm of water. c. For optimum condition of growth the soil should have 80 to 90% water saturation capacity.	

SAGE

Growing Season	Climate	Soil	Yield/Acre
1. Sage is a shrub. 2. It persists for several years. 3. It is propagated by cutting or seeds at a distance of 15 to 30 cm in the row of 61 to 92 cm. The seeds should be 2 cm deep.	1. The plant needs a sunny temperate climate. 2. It withstands winter temperatures to 15 below zero provided some protection is offered by snow or covering of leaves or straw. When exposed to temperature below -15°C or to winter winds, the plant is subjected to winterkill. 3. High summer temperatures and long dry periods are not conducive to good growth and quality of herb. 4. Where winters are severe enough to kill the plant, it may be grown as an annual plant. 5. Frost destroys the good aroma and the taste of the leaf.	1. The plant thrives best on a rich clay loam with a good drainage. 2. It does not thrive sufficiently well on a very light, sandy soil. 3. An excessive amount of moisture in the soil when subjected to freezing results in excessive winterkillings. 4. Heavy moist soil gives high yields of leaves but of inferior flavor. 5. Gravelly soil produces highly aromatic leaves.	1. First year 200 to 600 lb of dry leaves. 2. Second year 1,500 to 2,000 lb from 2 or 3 cuttings.

APPENDIX B: Infrared Spectra of Essential Oils Analyzed

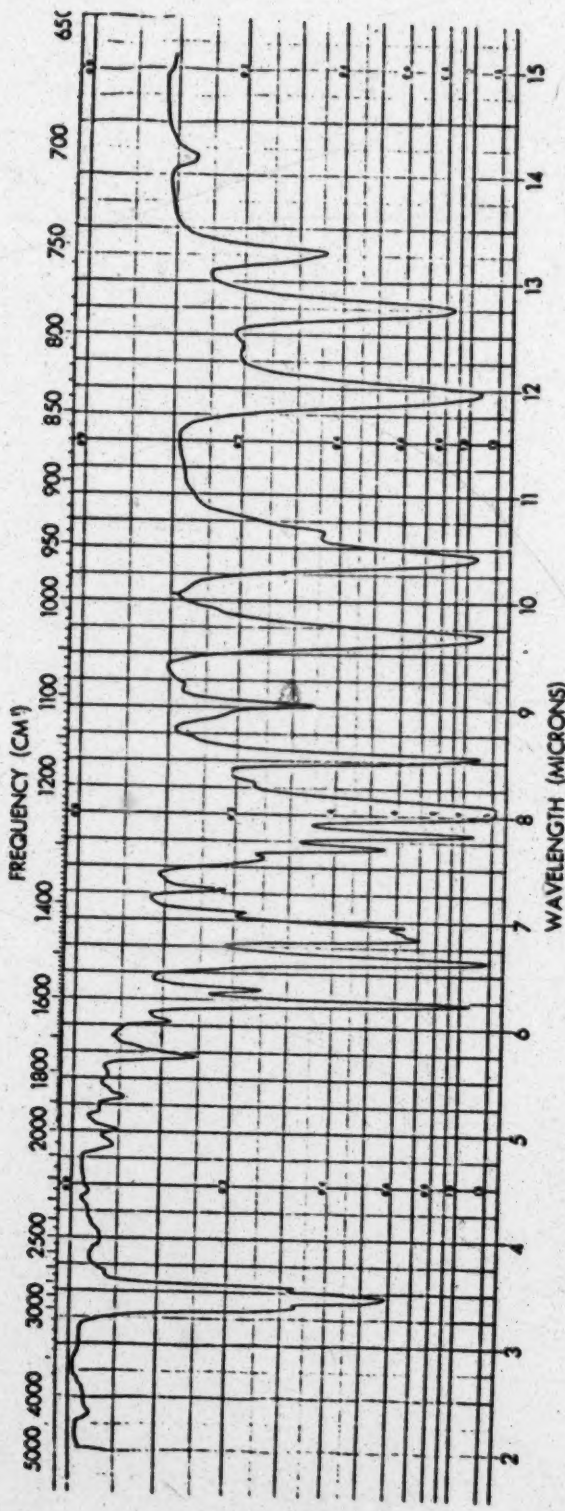


Fig. B-1a. Infrared Spectrum of Anise Seed Oil (Brooks--1972 Crop).

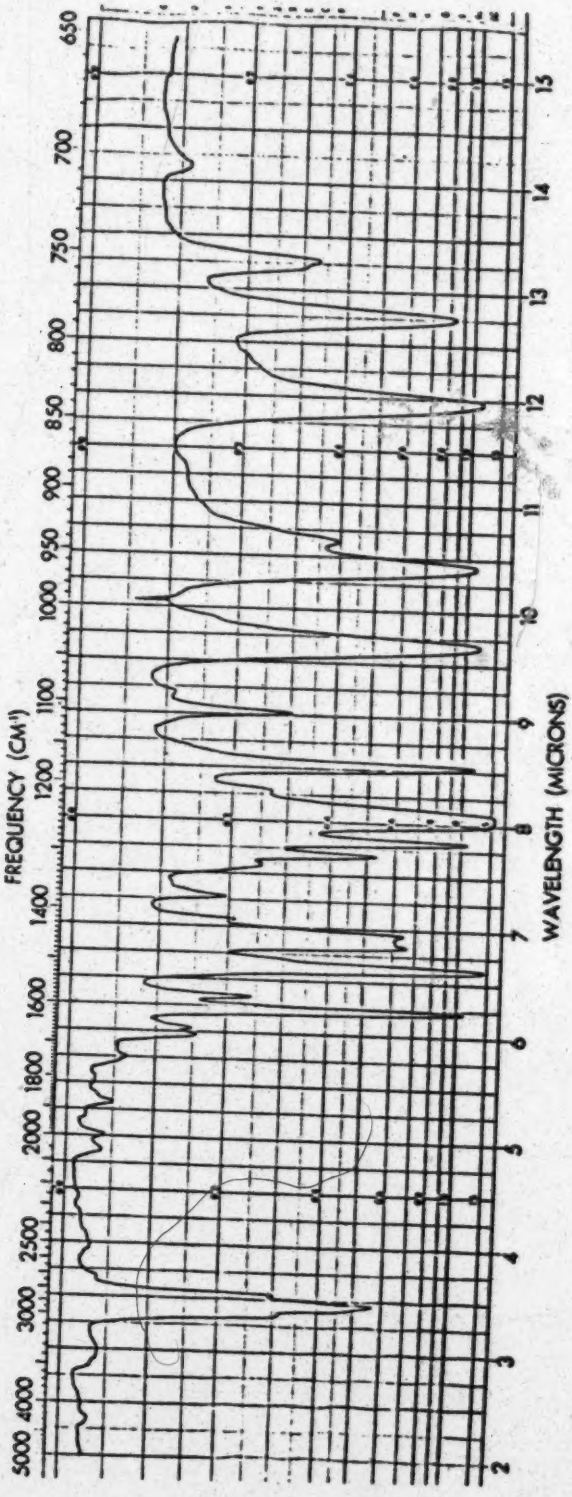


Fig. B-1b. Infrared Spectrum of Anise Seed Oil (Kalamazoo, Michigan).

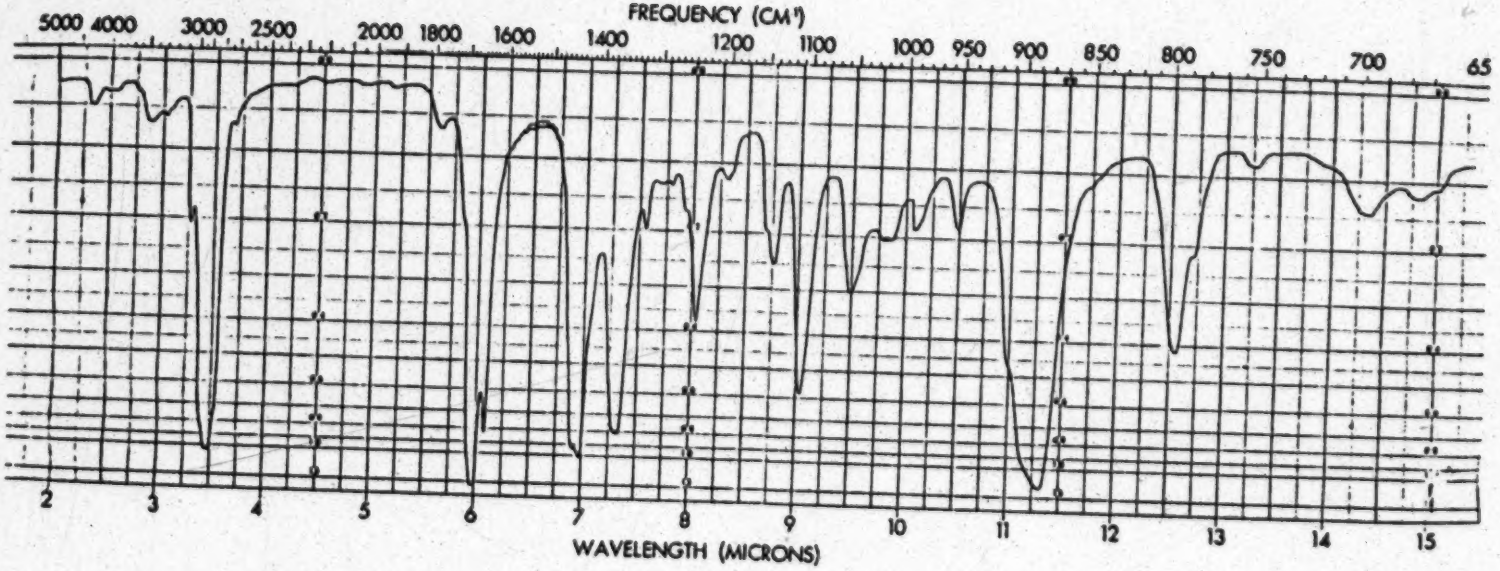


Fig. B-2. Infrared Spectrum of Caraway Seed Oil (Kalamazoo, Michigan).

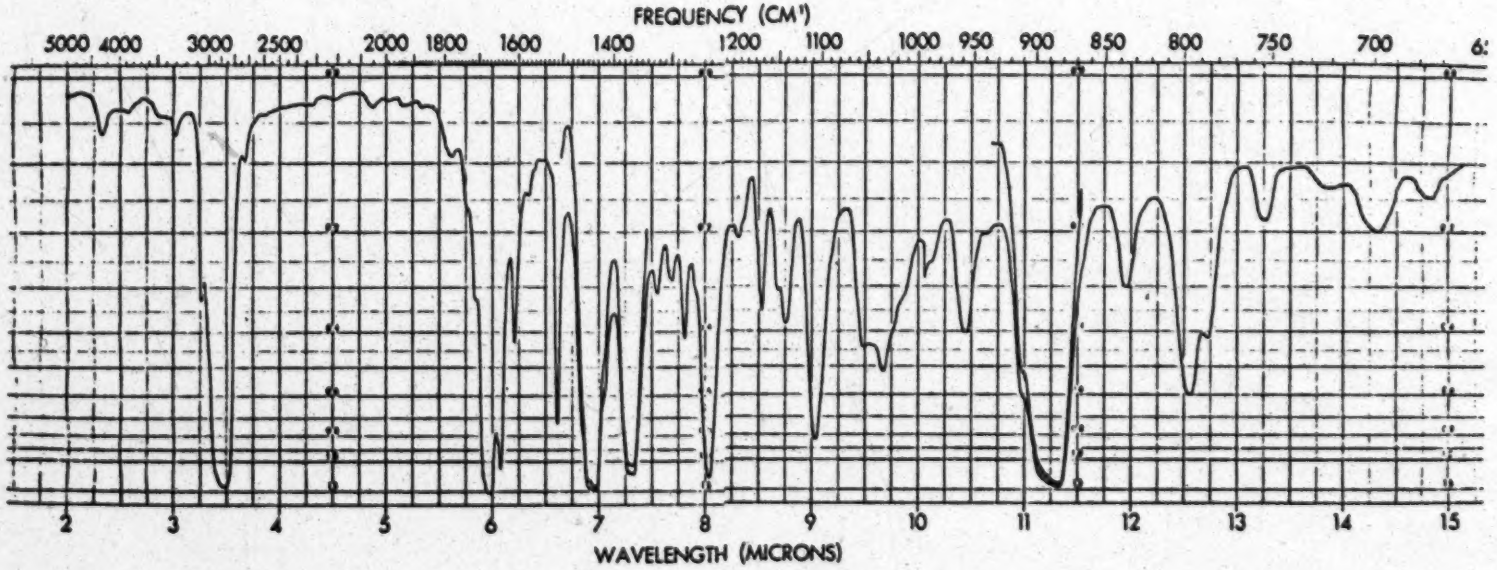


Fig. B-3a. Infrared Spectrum of Common Dill Seed Oil (Brooks--1971 Crop).

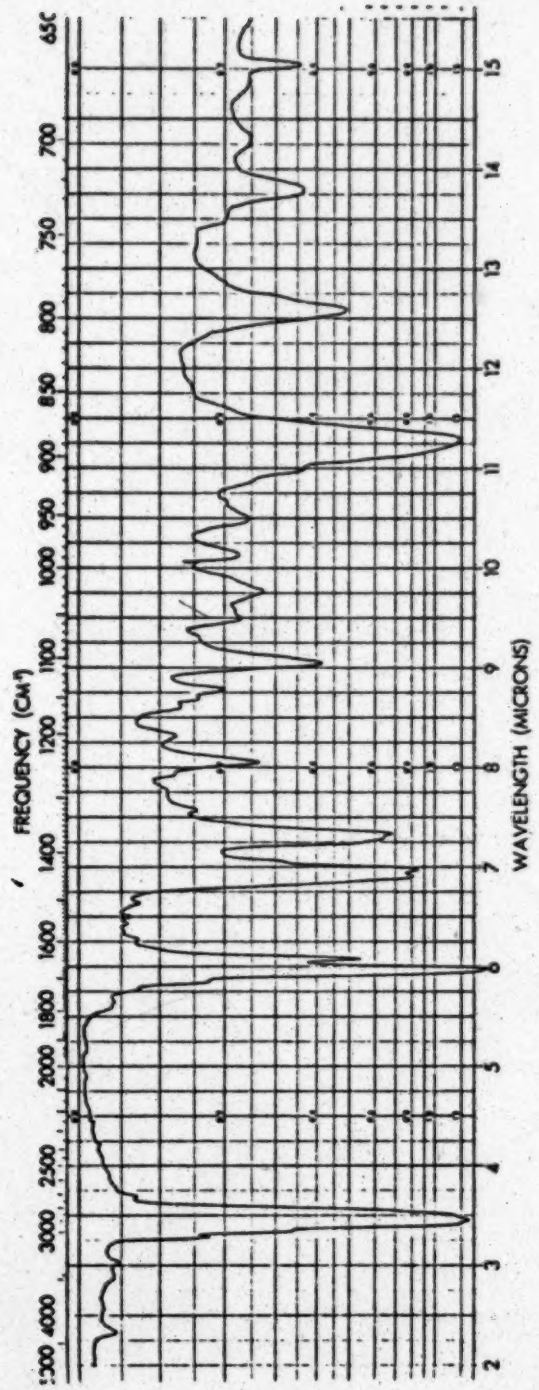


Fig. B-3b. Infrared Spectrum of D[1] Prime Oil (Kalamazoo, Michigan).

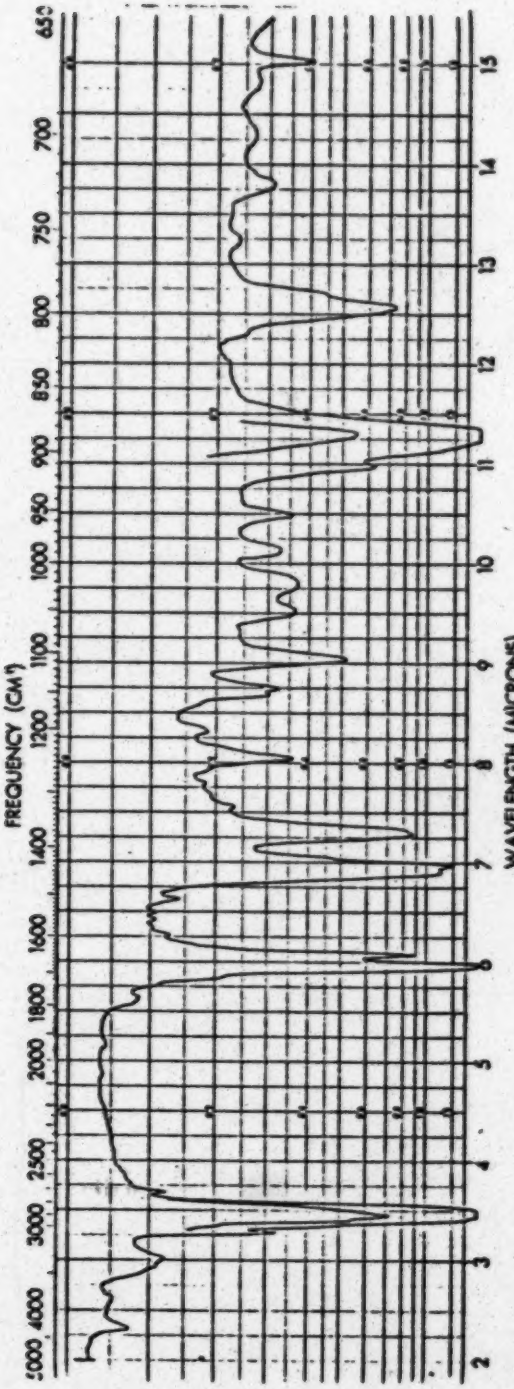


Fig. B-3c. Infrared Spectrum of D111 Standard 011 (Kalamazoo, Michigan).

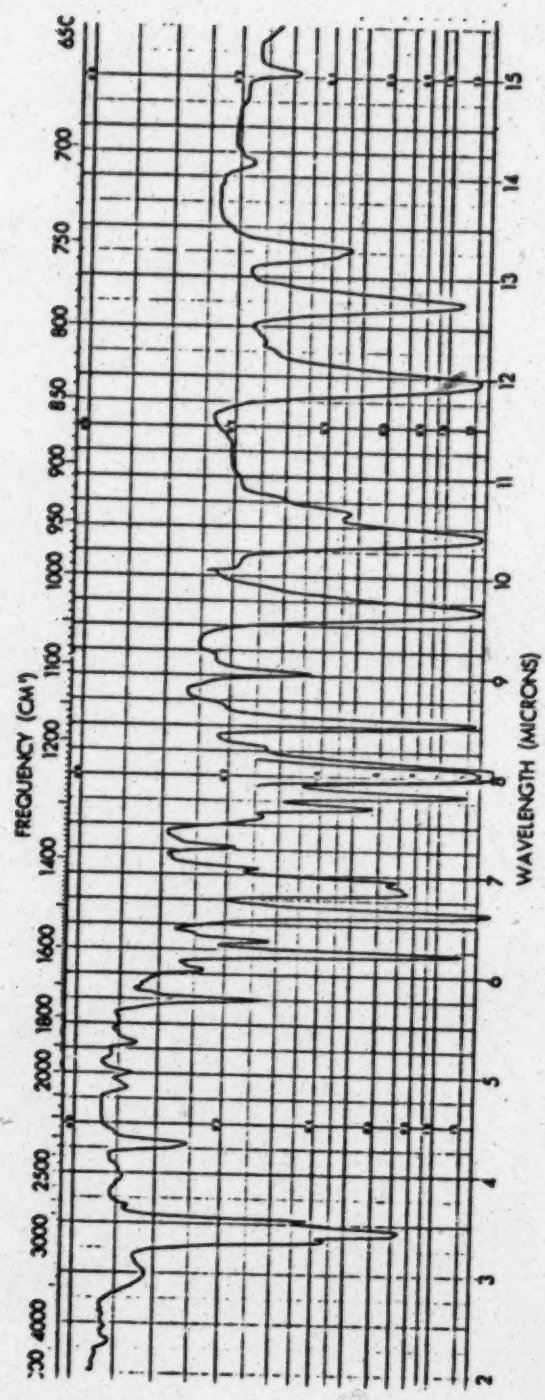


Fig. B-4a. Infrared Spectrum of Fennel Seed 011 (Brooks--1972 Crop).

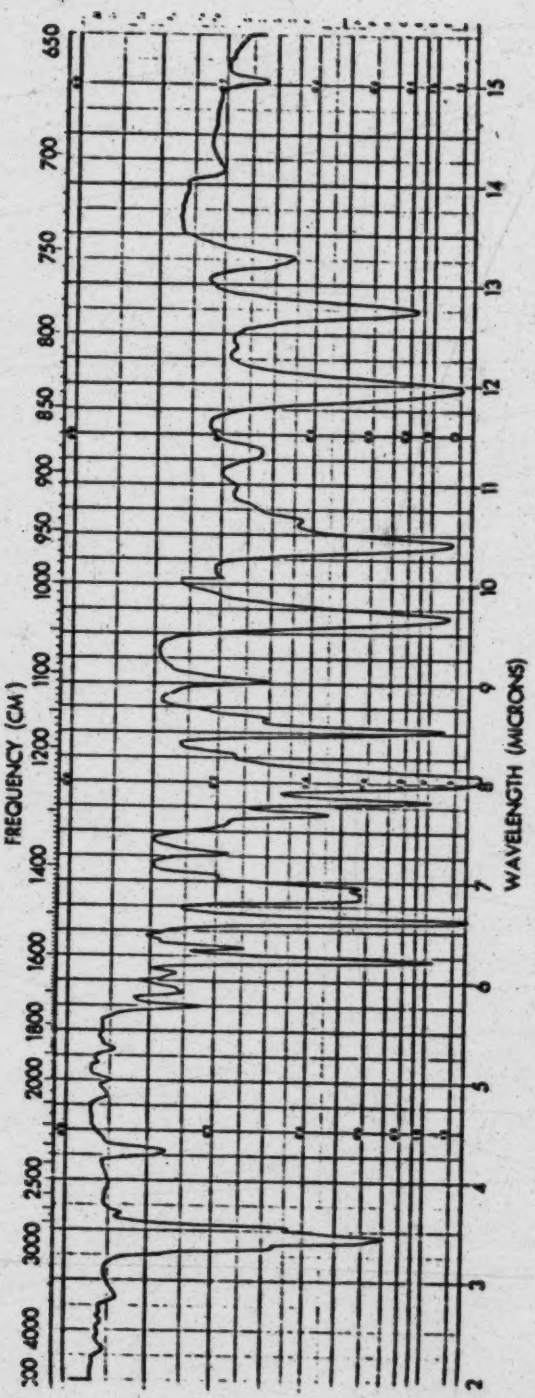


Fig. B-4b. Infrared Spectrum of Fennel Seed Oil (Kalamazoo, Michigan).

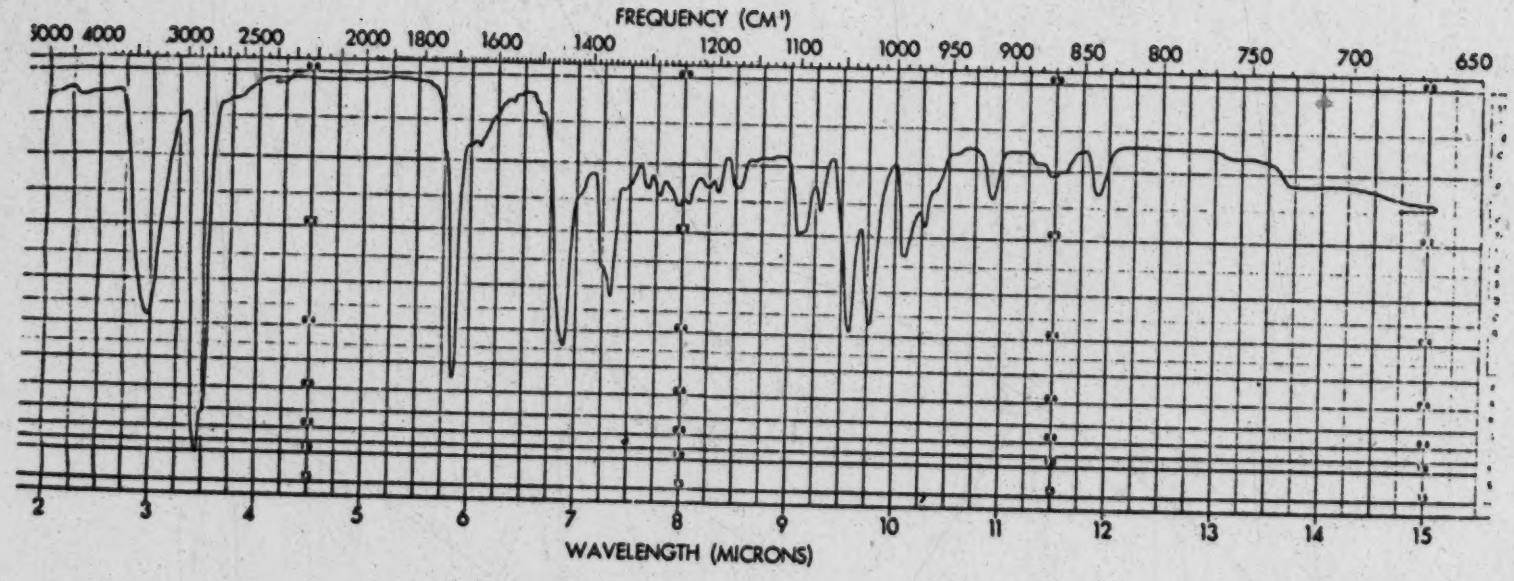


Fig. B-5a. Infrared Spectrum of Peppermint Oil (Leaf, Stage II, Brooks--1970 Crop).

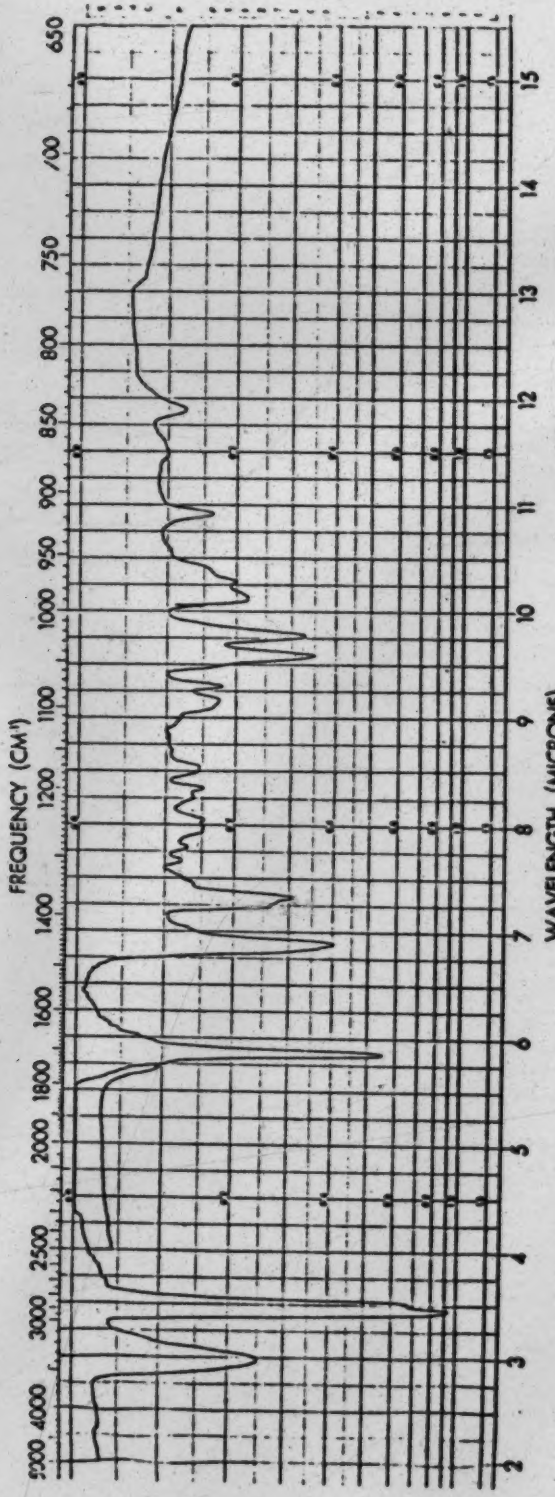


Fig. B-5b. Infrared Spectrum of Peppermint Oil (Leaf, Stage III, Brooks--1970 Crop).

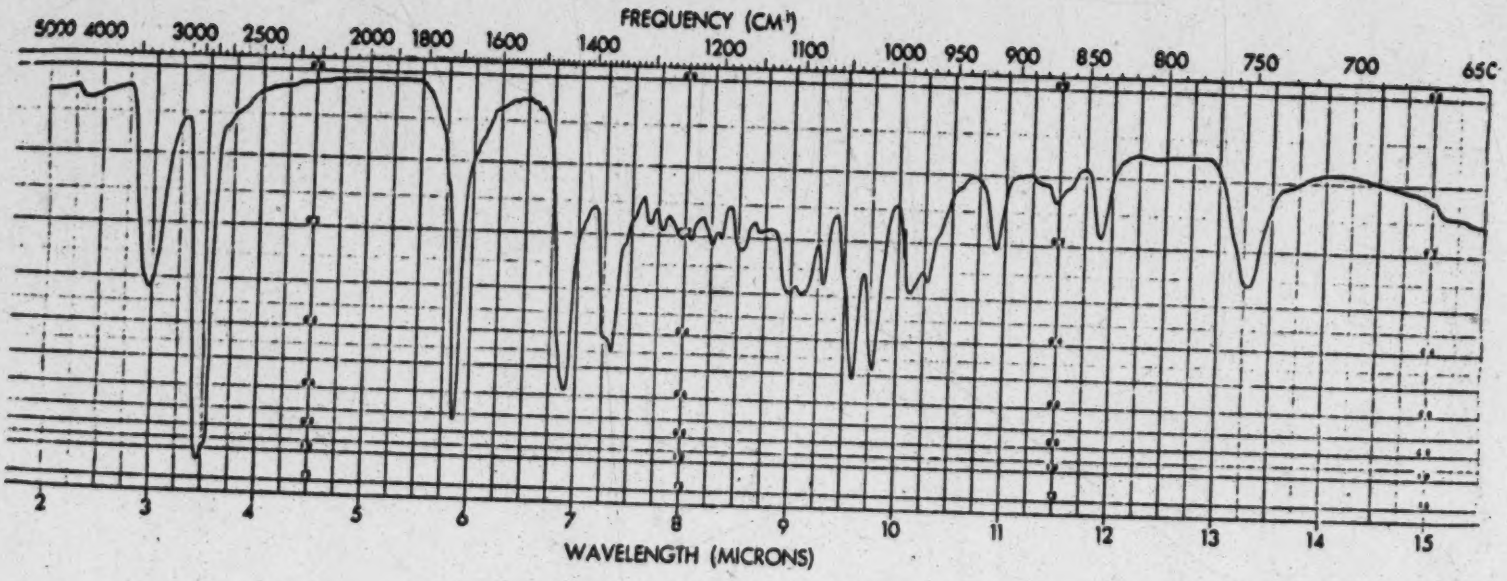


Fig. B-5c. Infrared Spectrum of Peppermint Oil (Leaf, Stage IV, Brooks--1970 Crop).

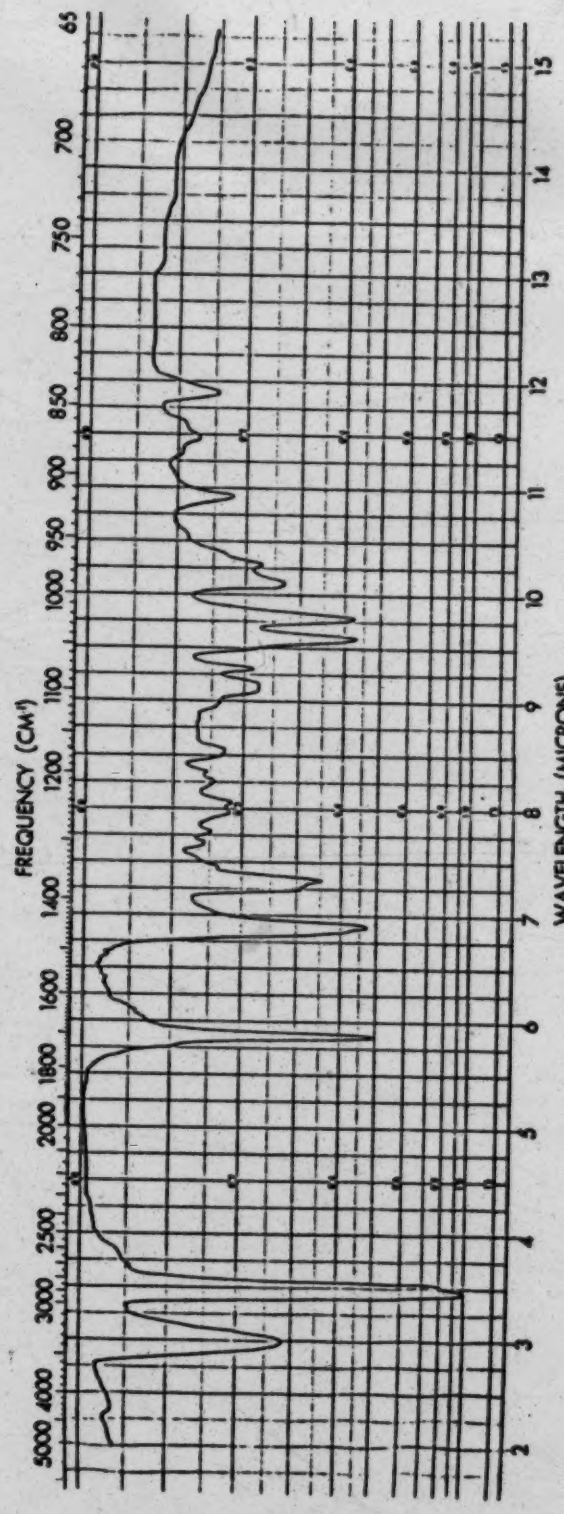


Fig. B-5d. Infrared Spectrum of Peppermint Oil (Leaf, Stage V, Brooks--1970 Crop).

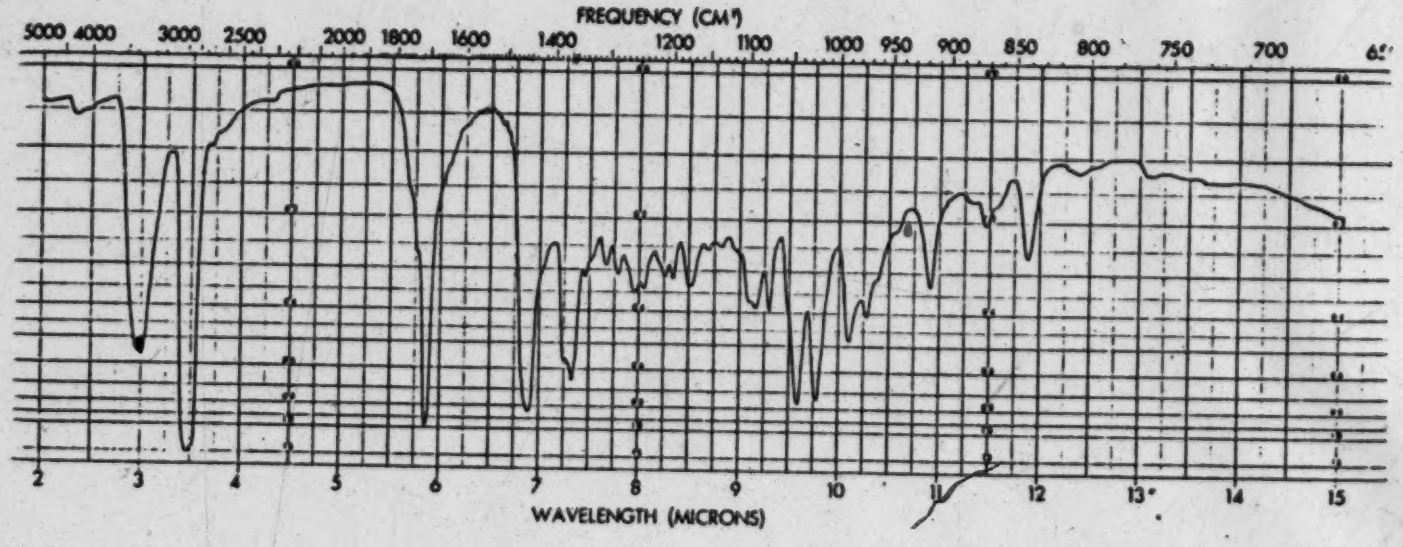


Fig. B-5e. Infrared Spectrum of Peppermint Oil (Stem, Stage I, Brooks--1970 Crop).

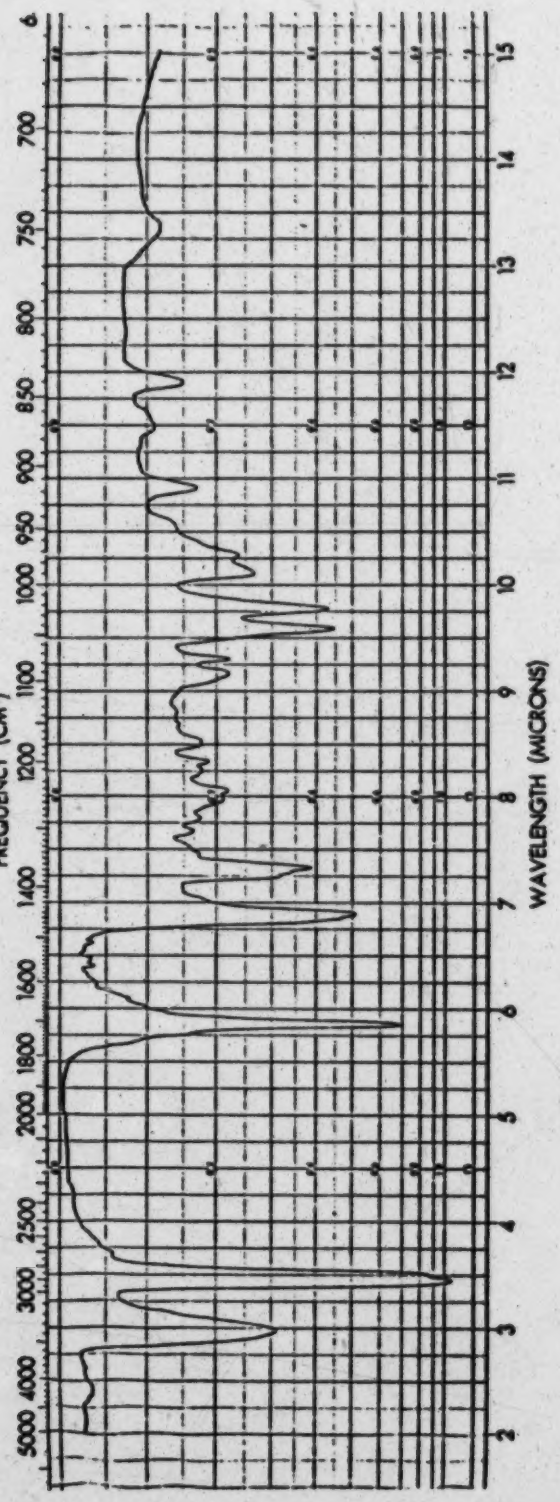


Fig. B-5f. Infrared Spectrum of Peppermint Oil (Stem, Stage III, Brooks--1970 Crop).

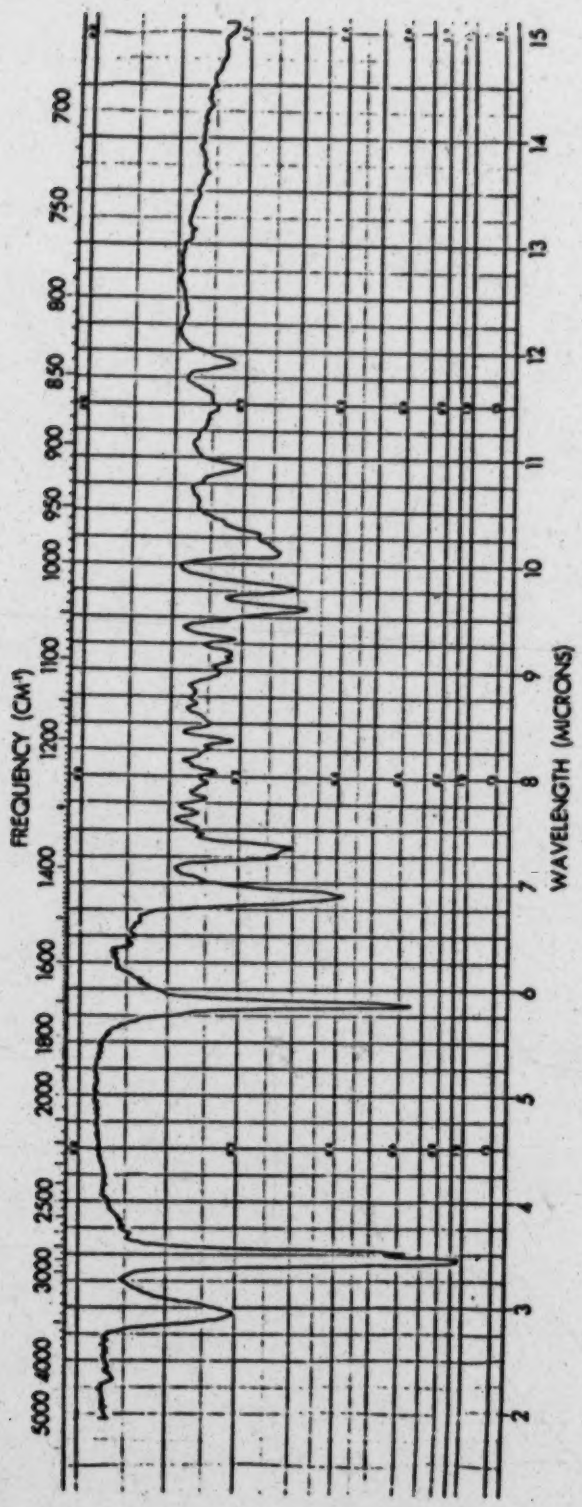


Fig. B-5g. Infrared Spectrum of Peppermint Oil (Michigan).

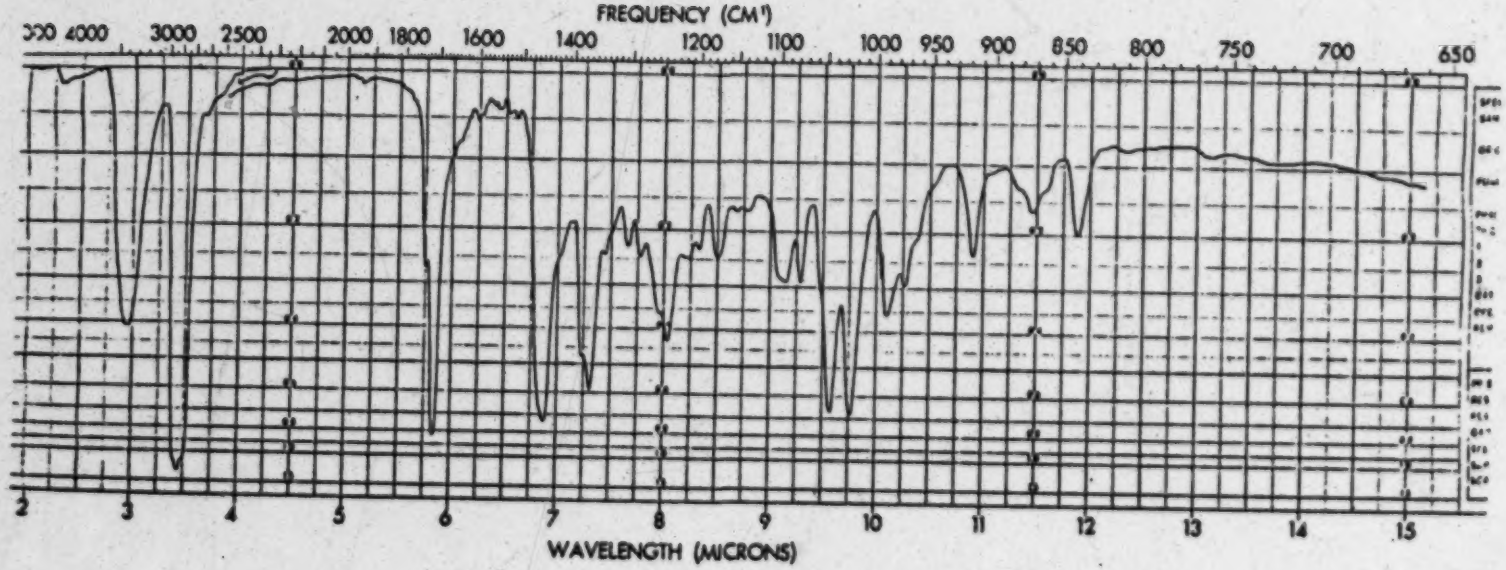


Fig. B-5h. Infrared Spectrum of Peppermint Oil (Michigan).

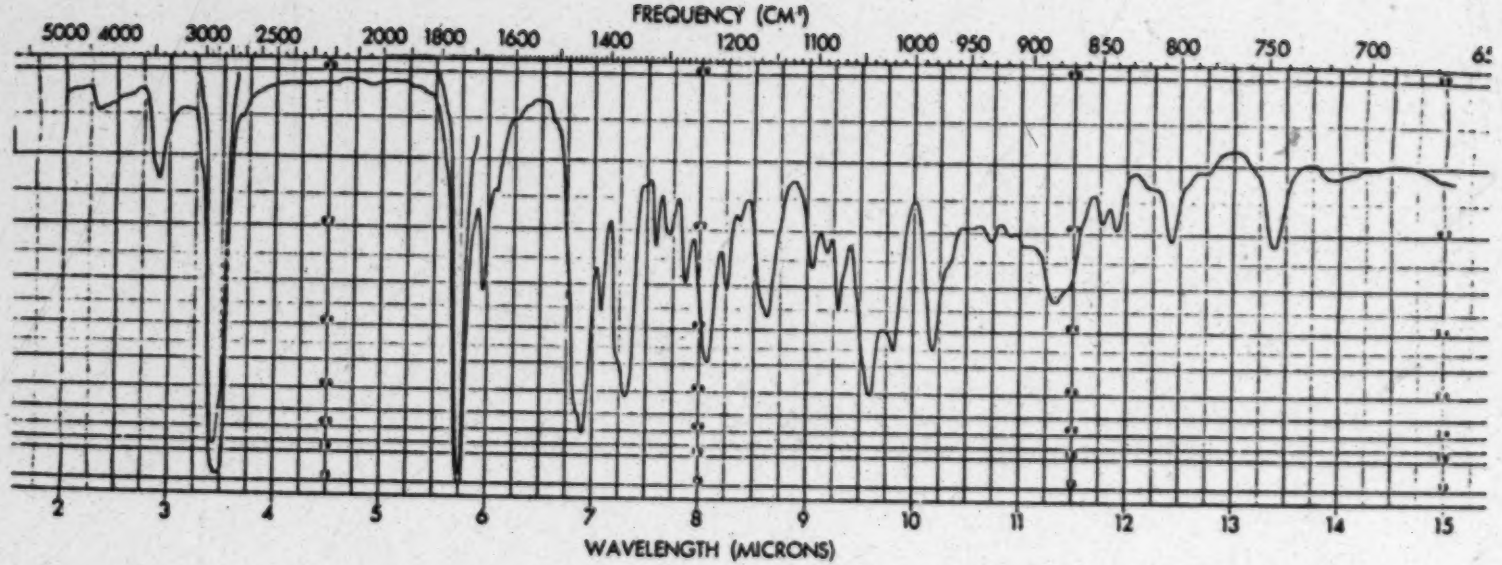
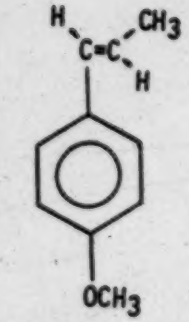
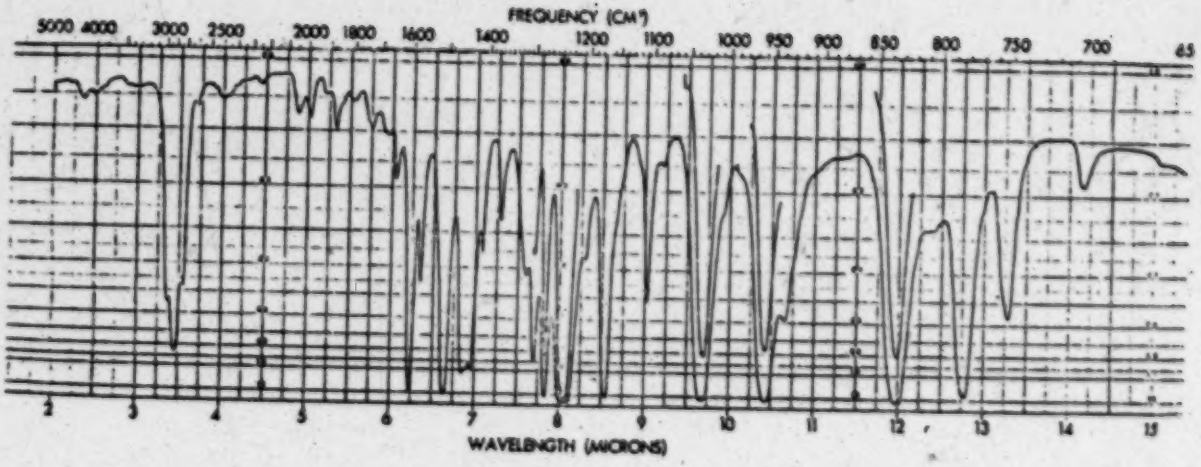


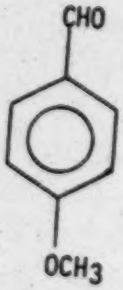
Fig. B-6. Infrared Spectrum of Sage 011 (Kalamazoo, Michigan).

APPENDIX C: Infrared Spectra of Pure Compounds Present in Essential Oils Studied

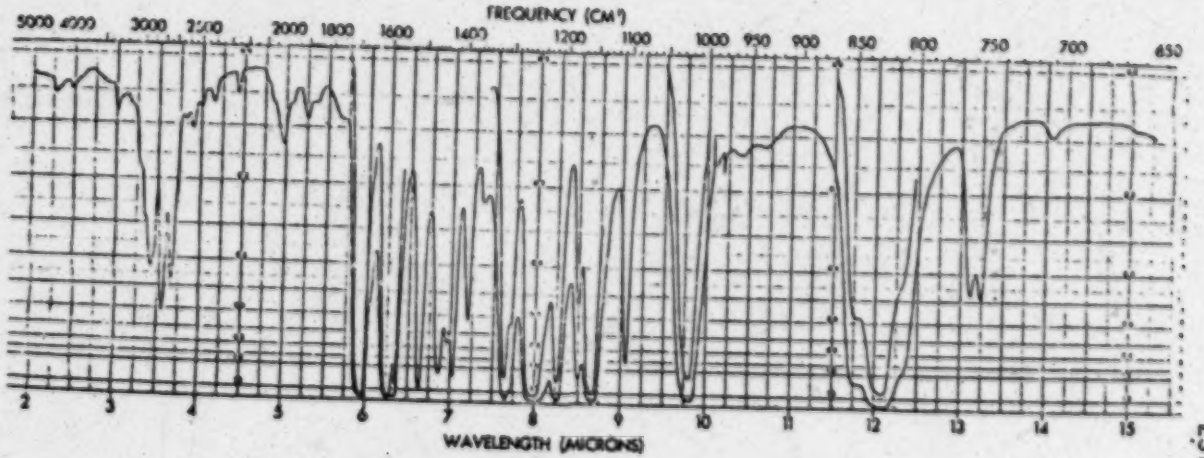


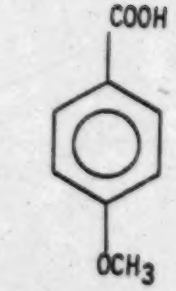
trans-Anethole



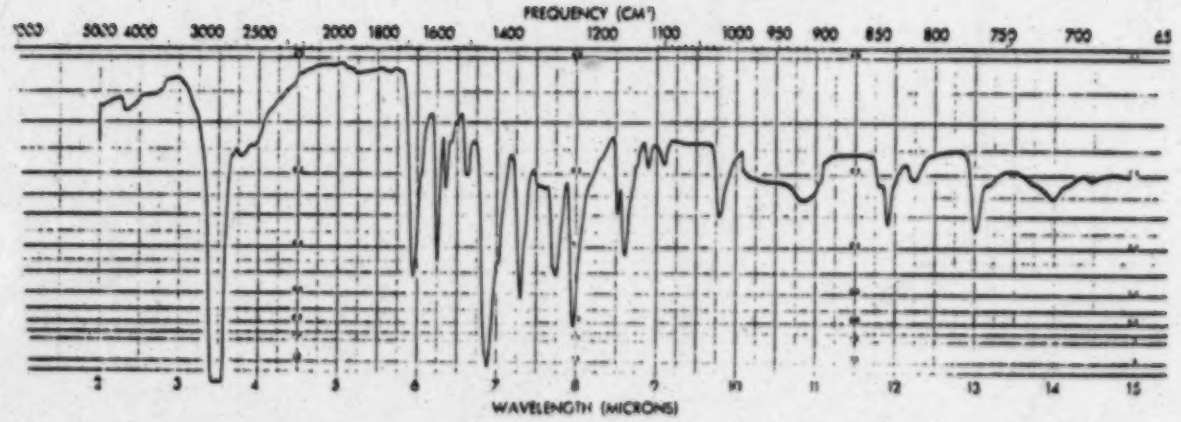


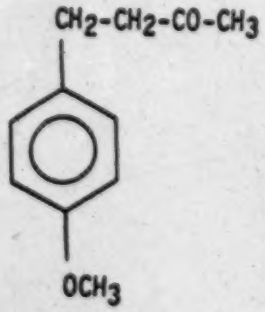
p-Anisaldehyde



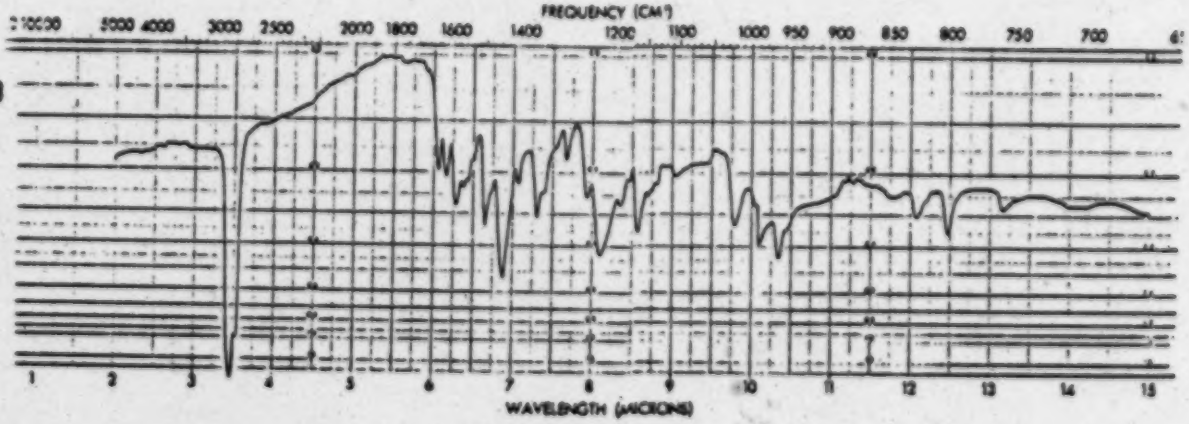


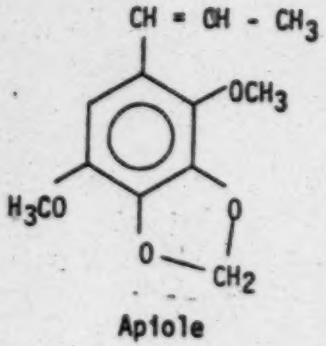
p-Anisic acid



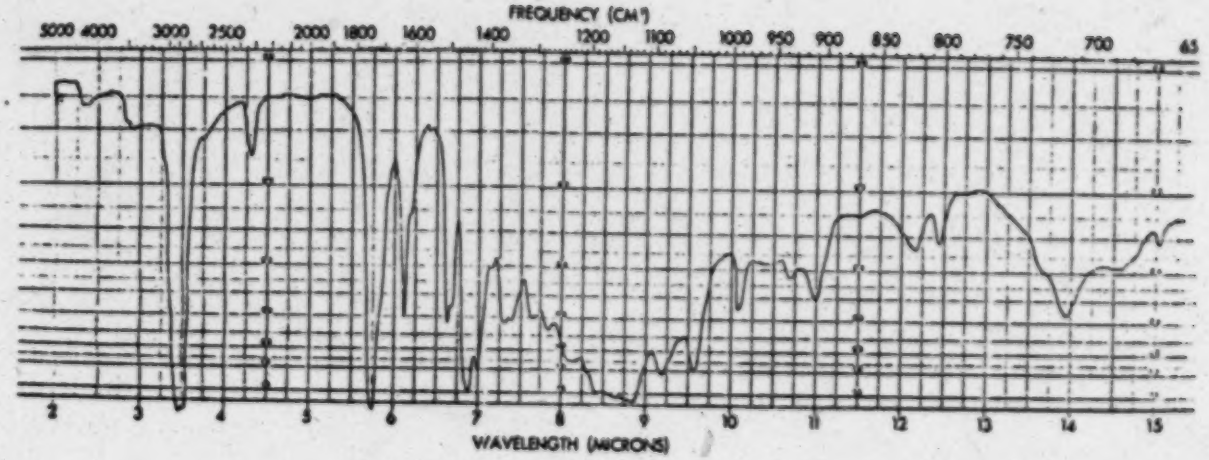


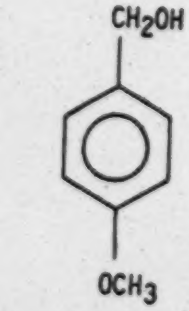
Anisyl acetone



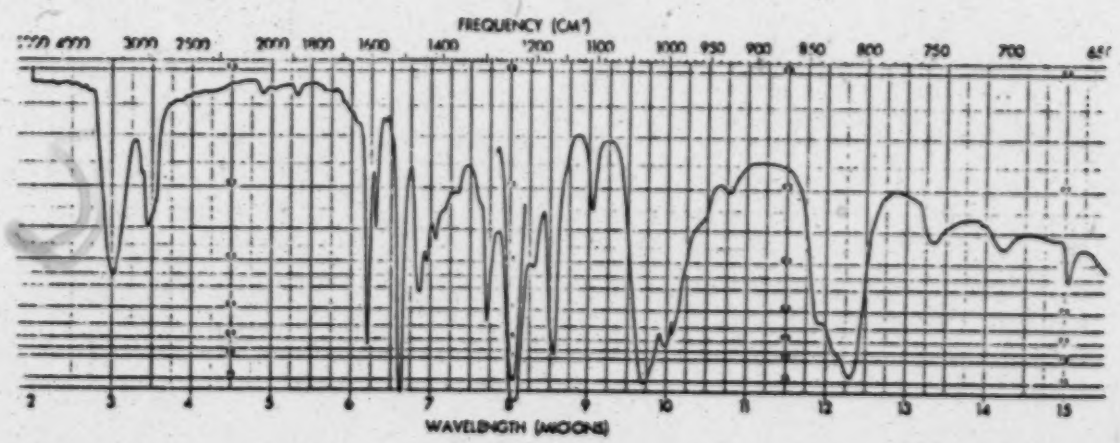


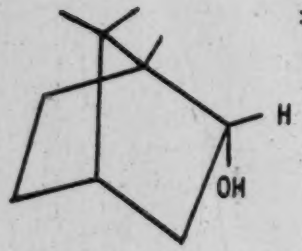
Apiole



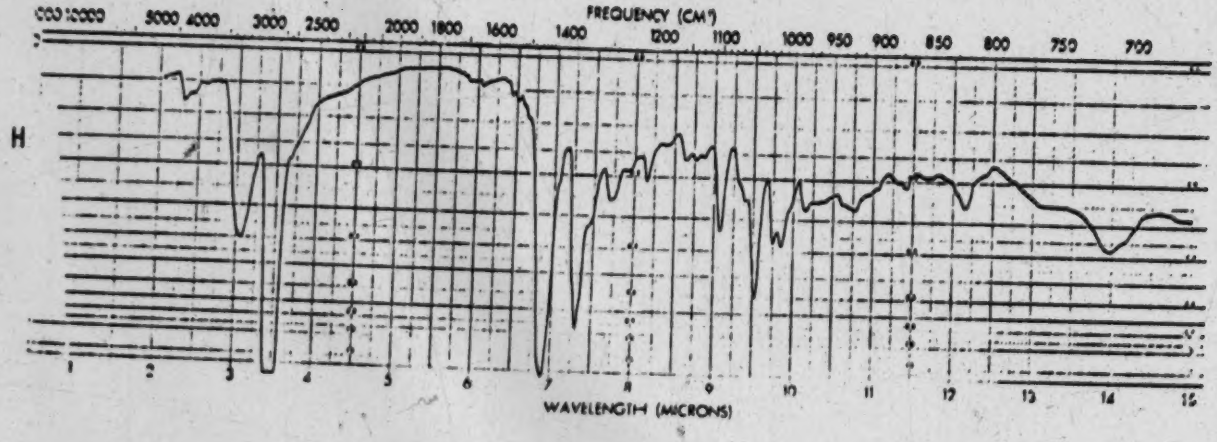


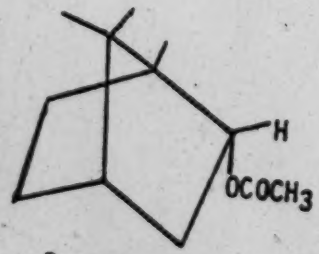
Anisyl alcohol





Borneol





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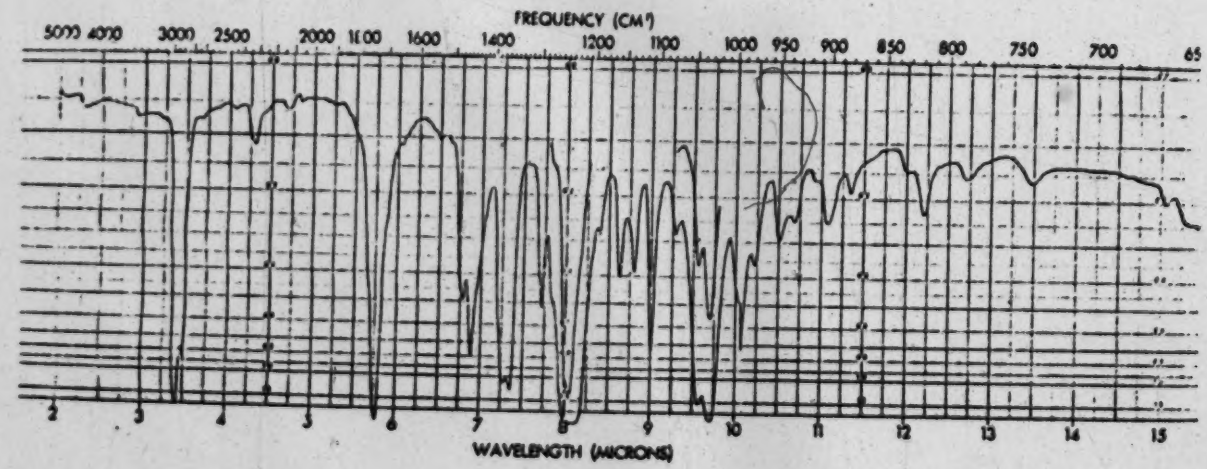
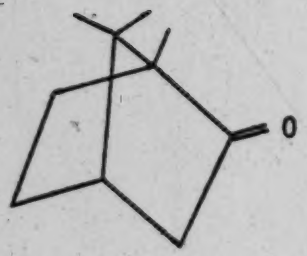
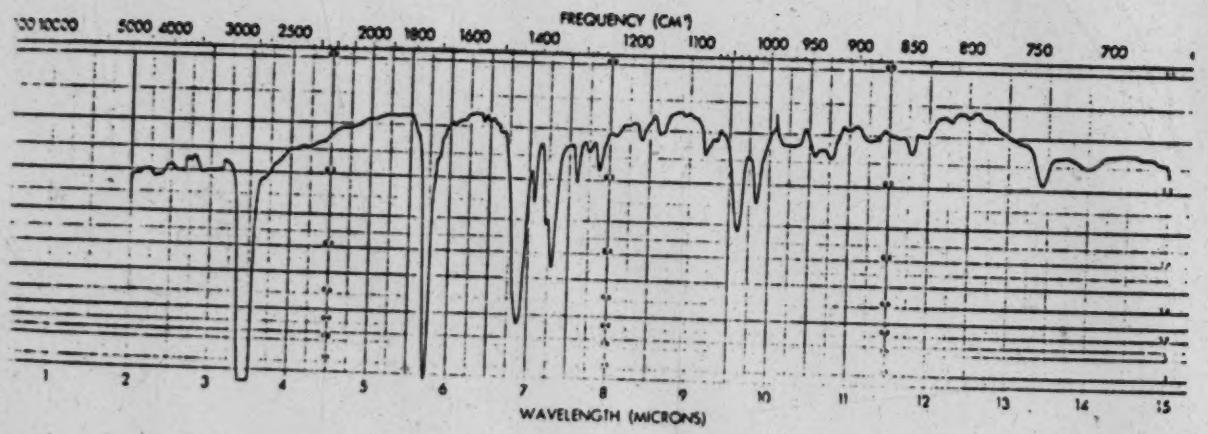




Camphor



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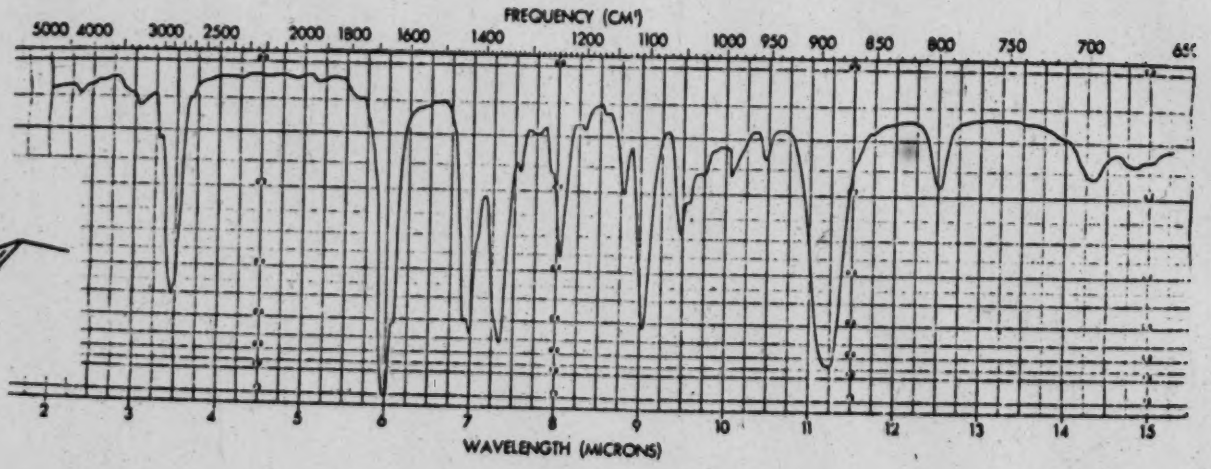
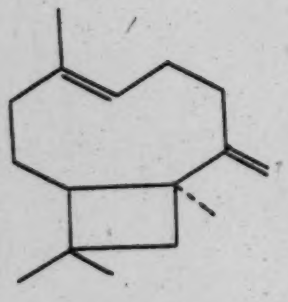
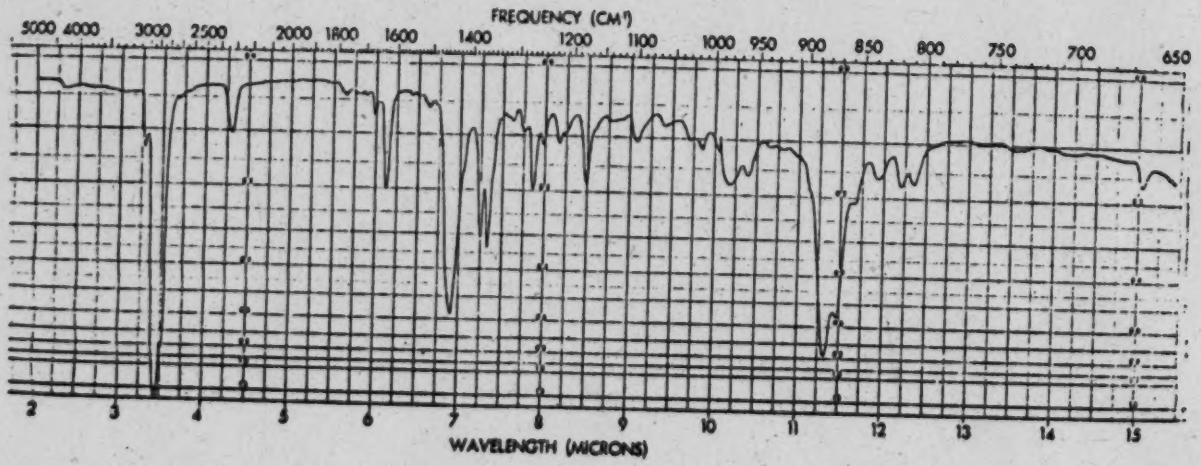
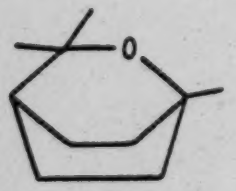


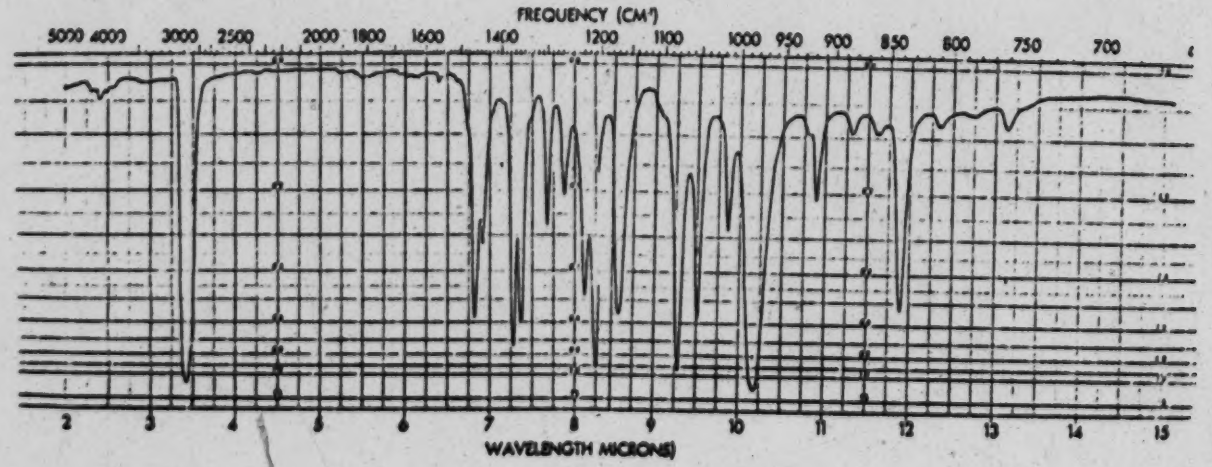


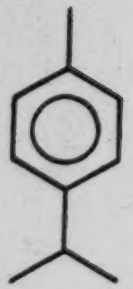
β-Caryophyllene



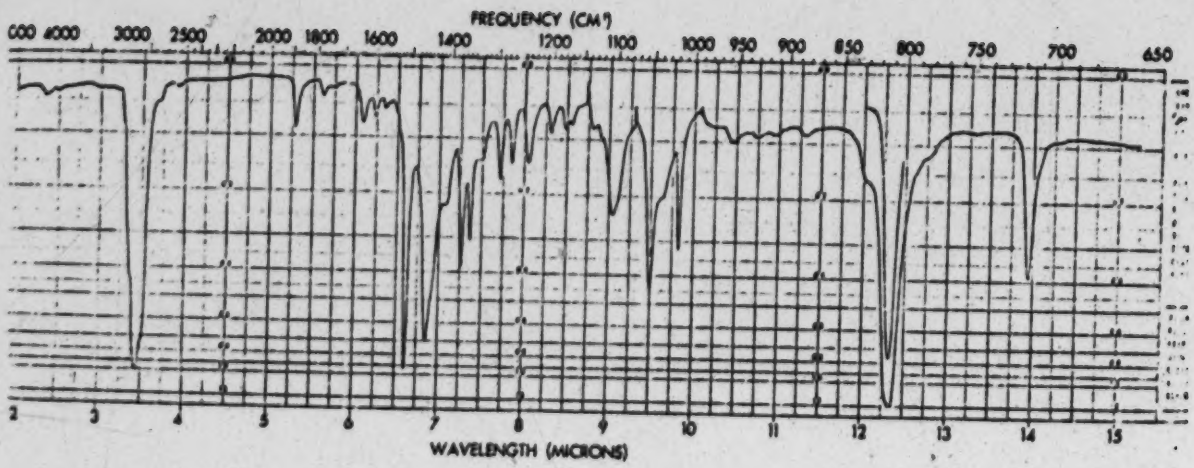


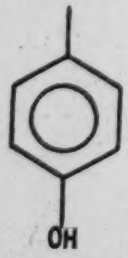
1,8-Cineol



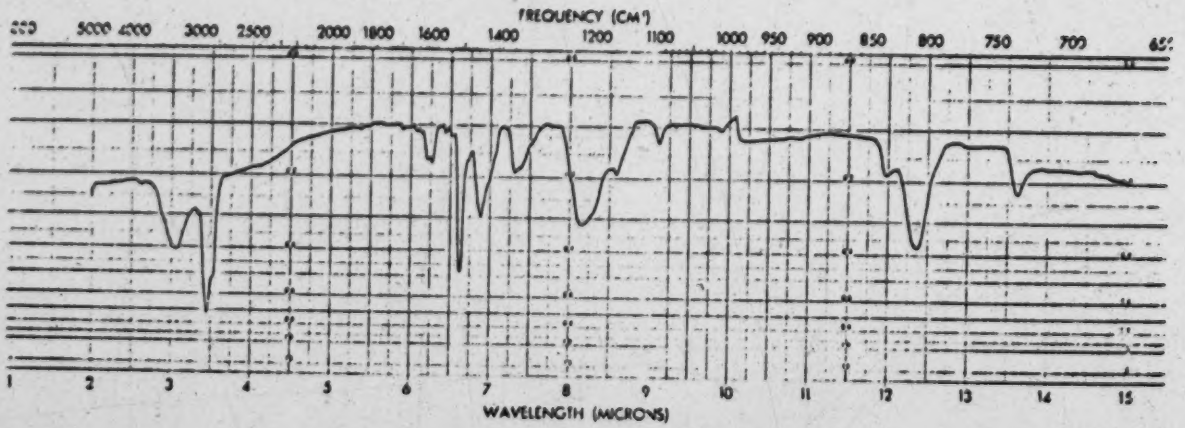


p-Cymene

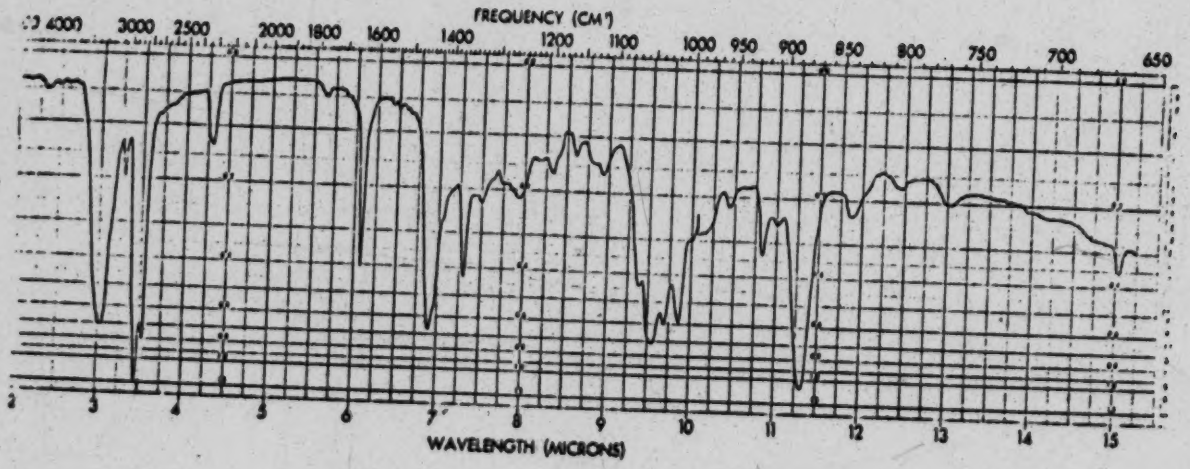




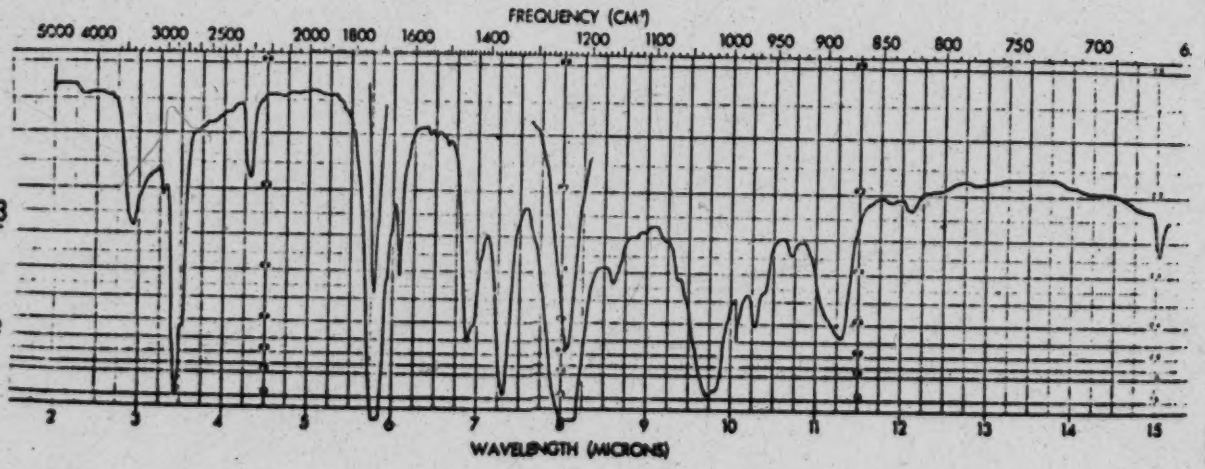
p-Cresol

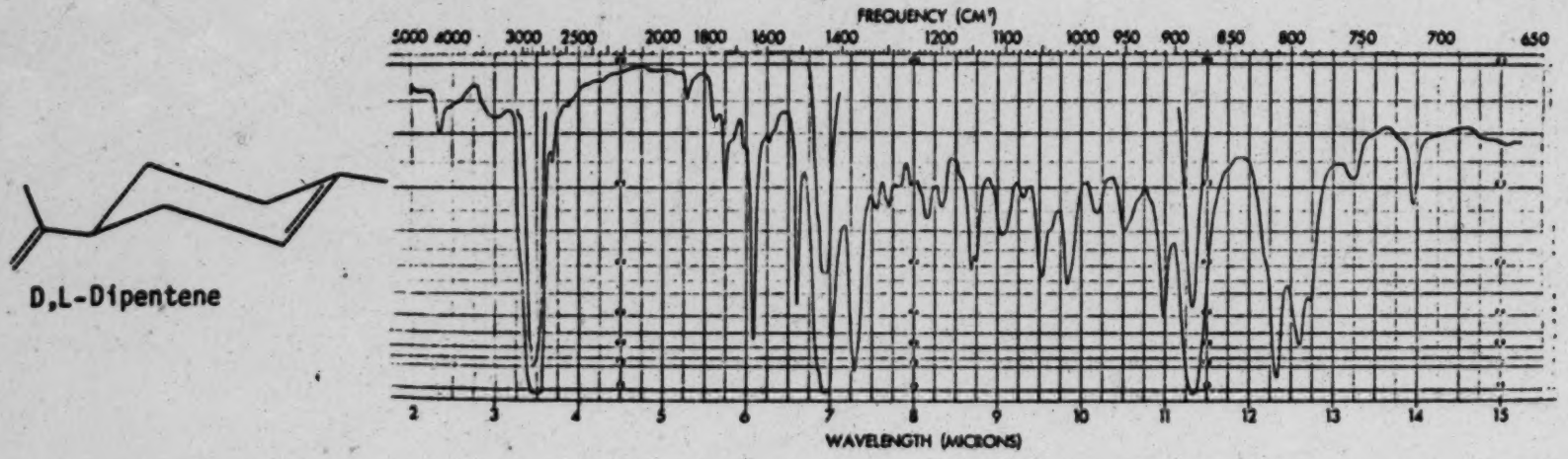


Dihydrocarveol

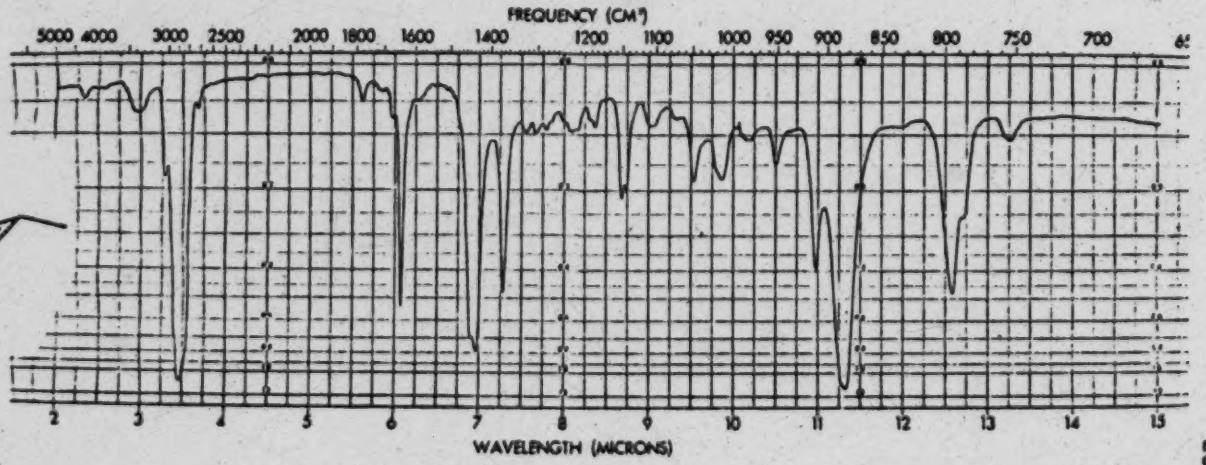


Dihydrocarvyl acetate

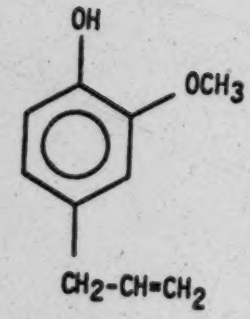




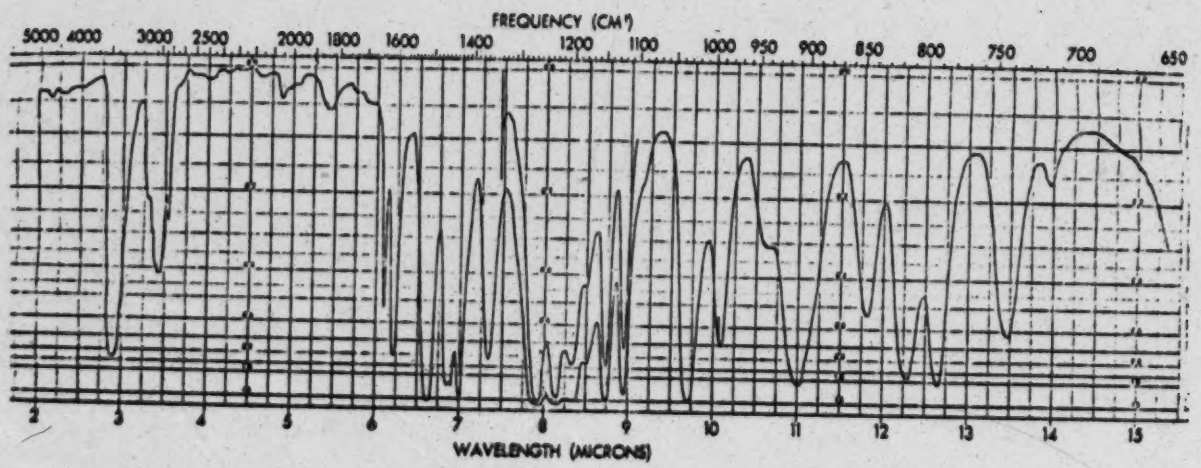
D,L-Dipentene

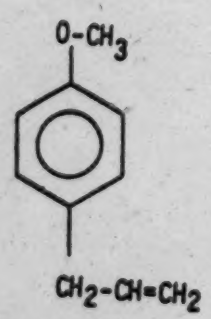


D(+)-Limonene

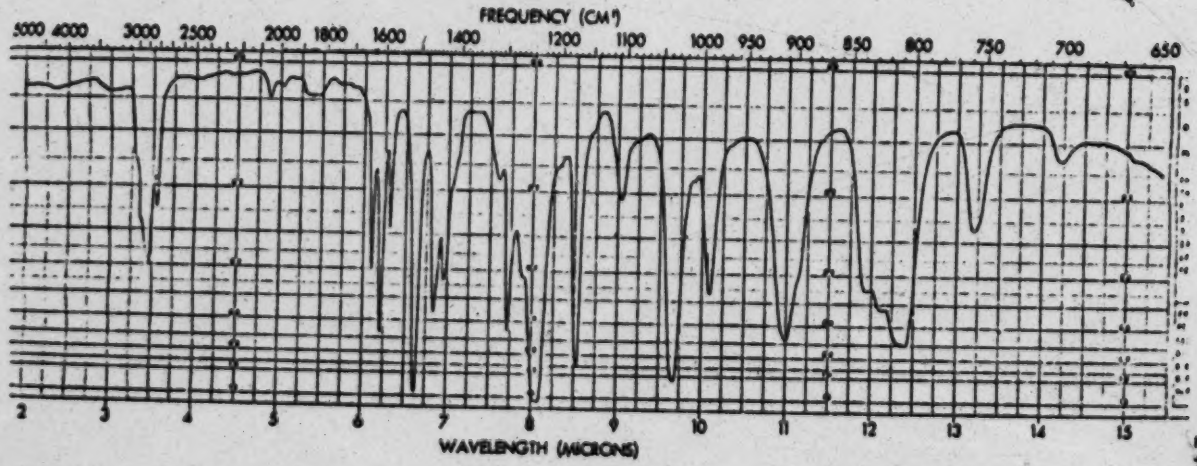


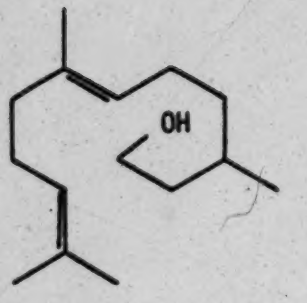
Eugenol



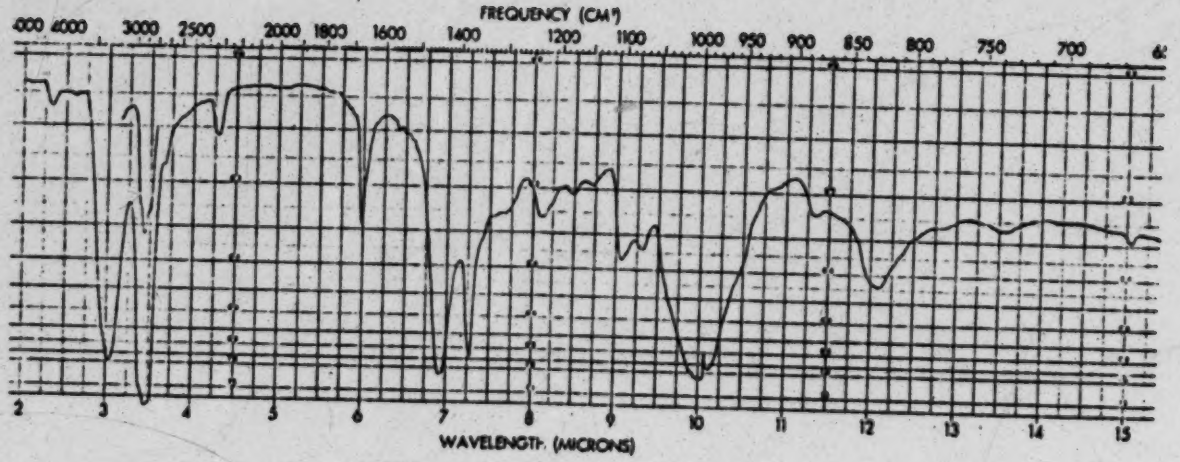


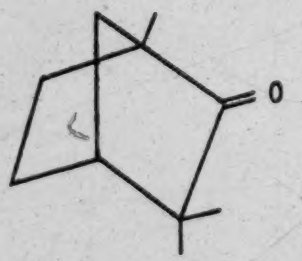
Estragole





Farnesol





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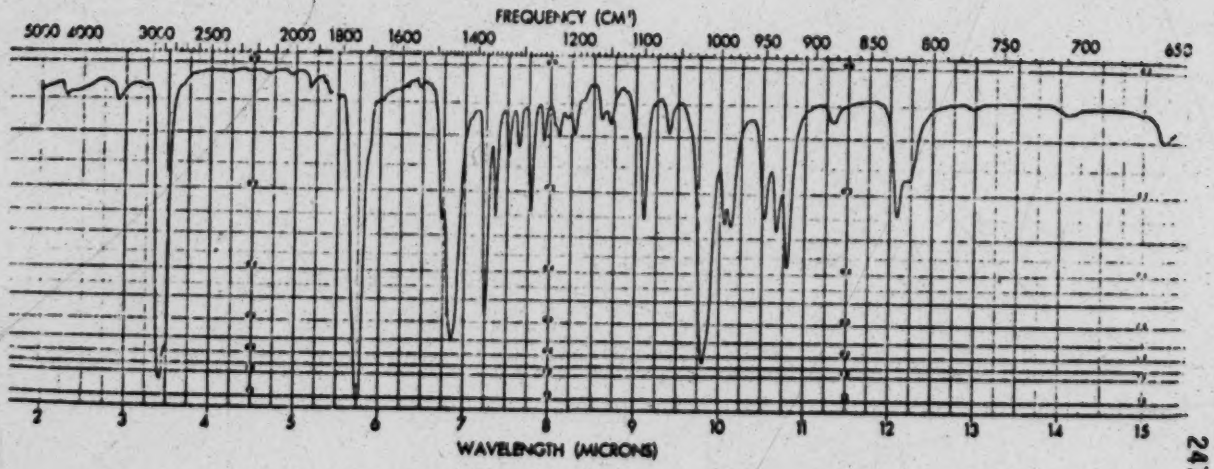
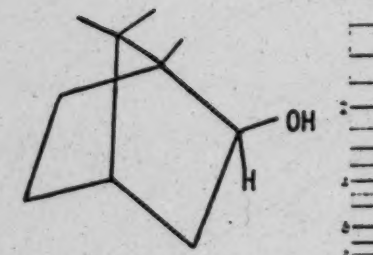
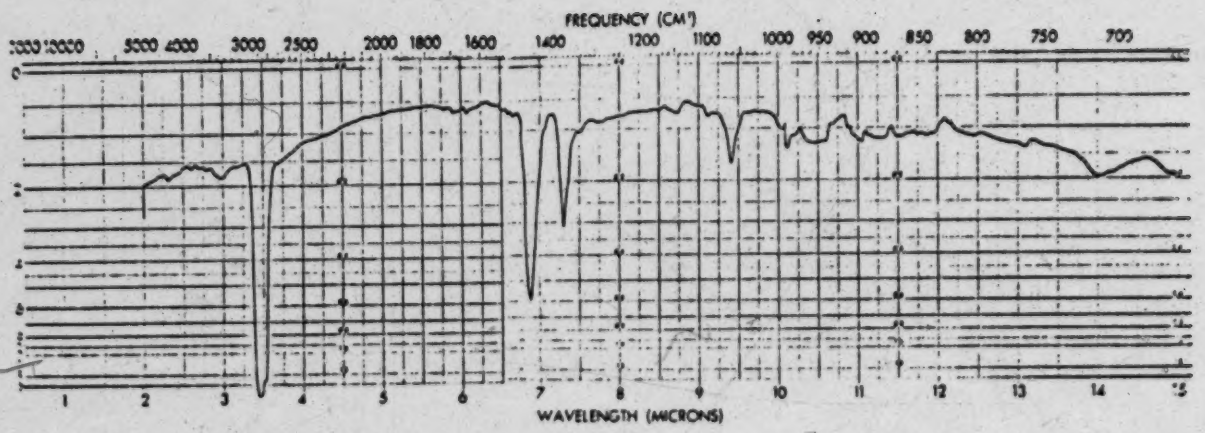
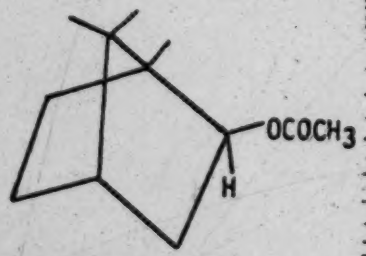


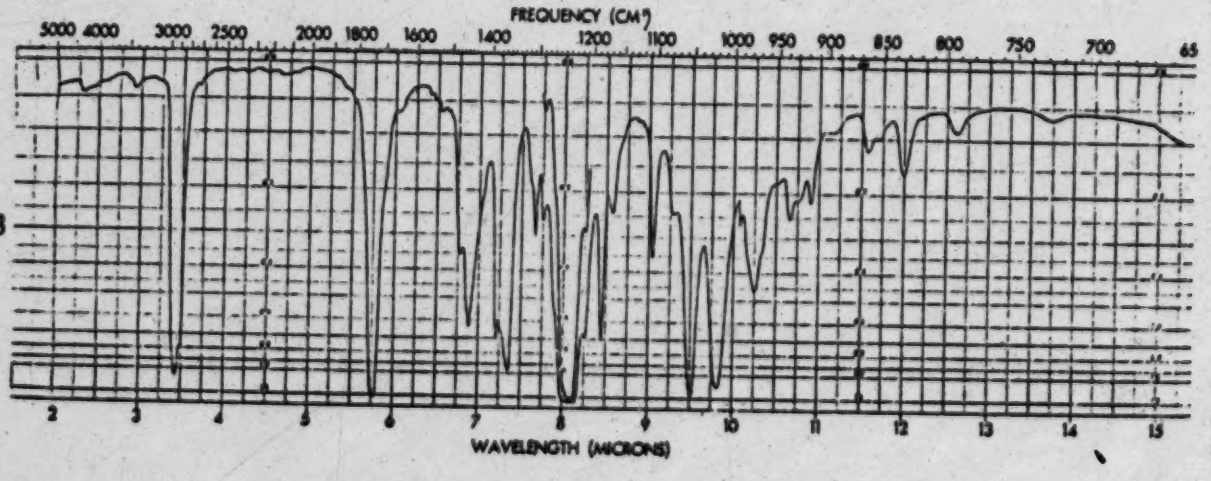


Isoborneol

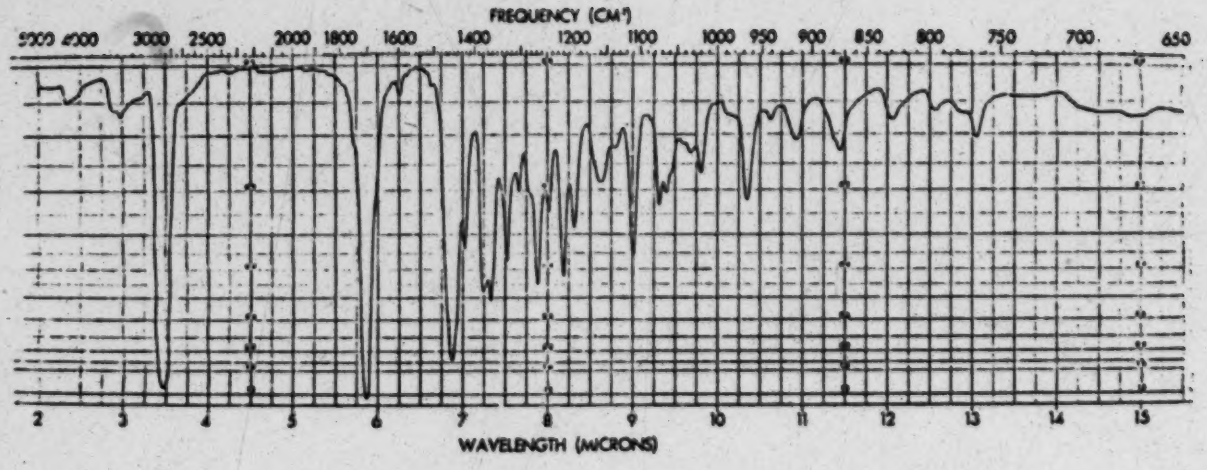




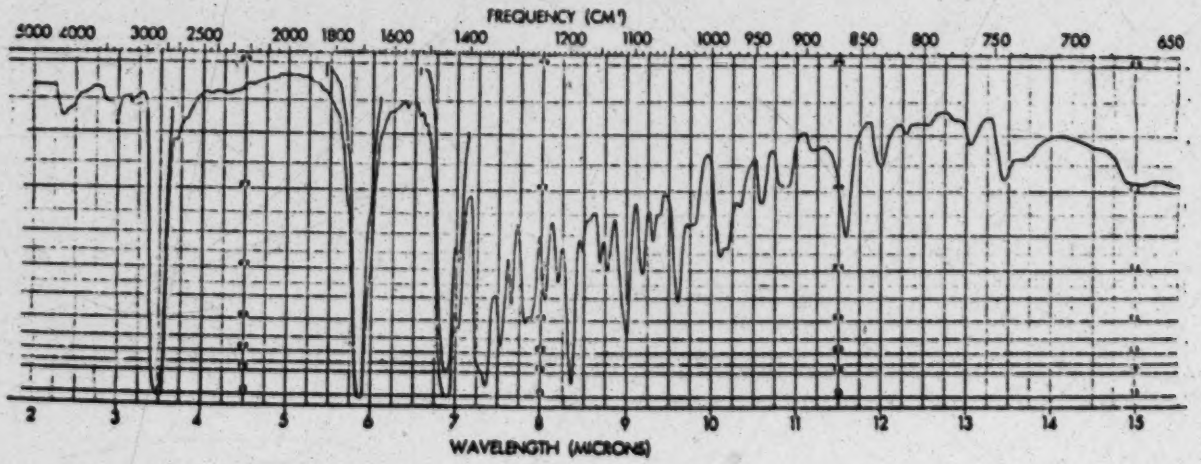
Isobornyl acetate

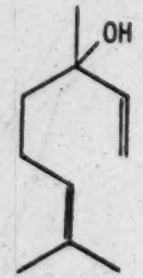


Isomenthone

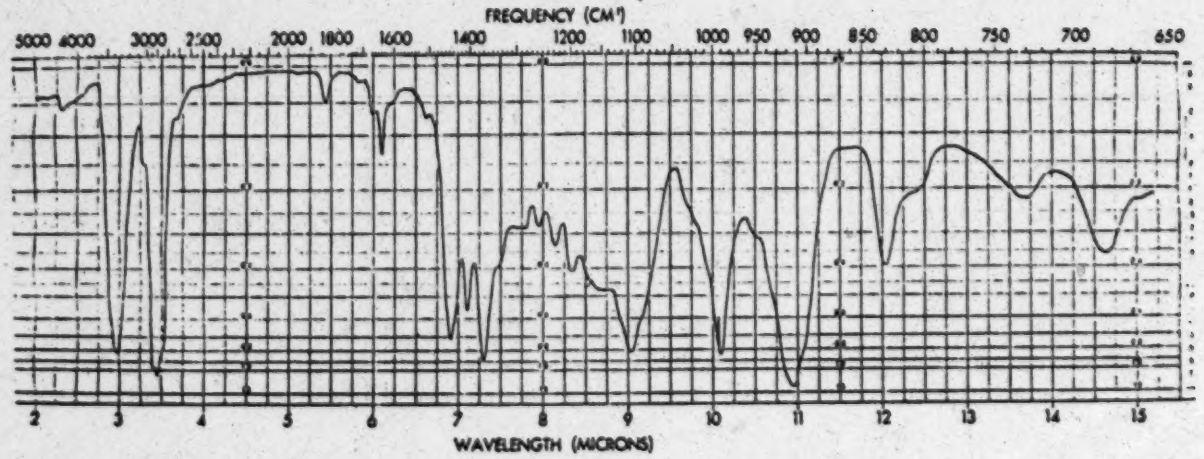


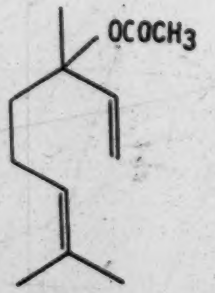
Menthone



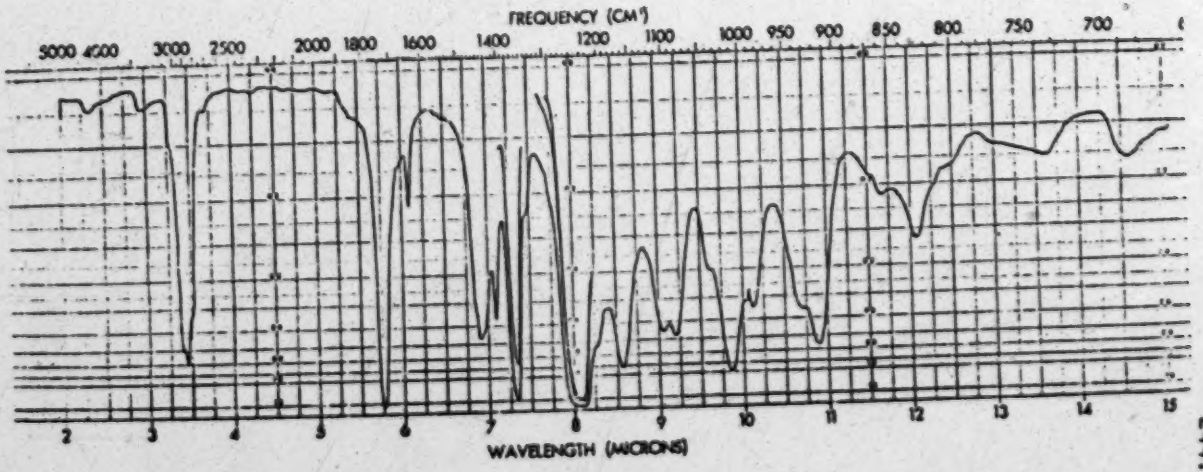


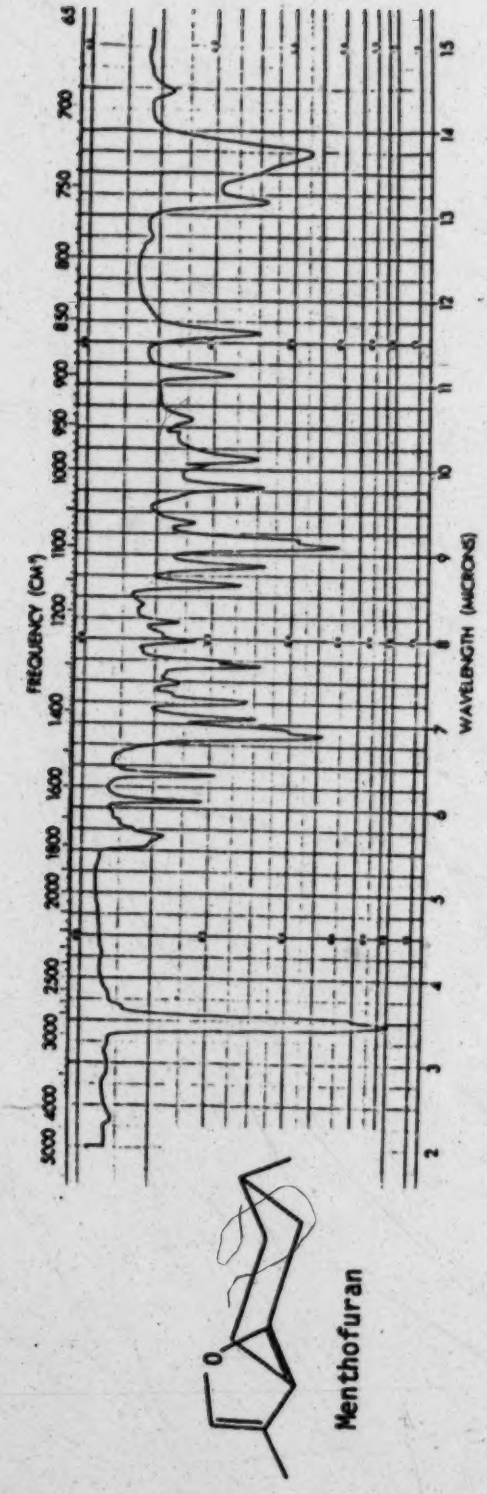
Linalool



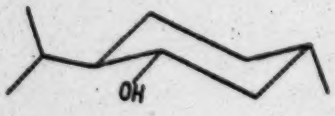


Linalyl acetate





Menthofuran



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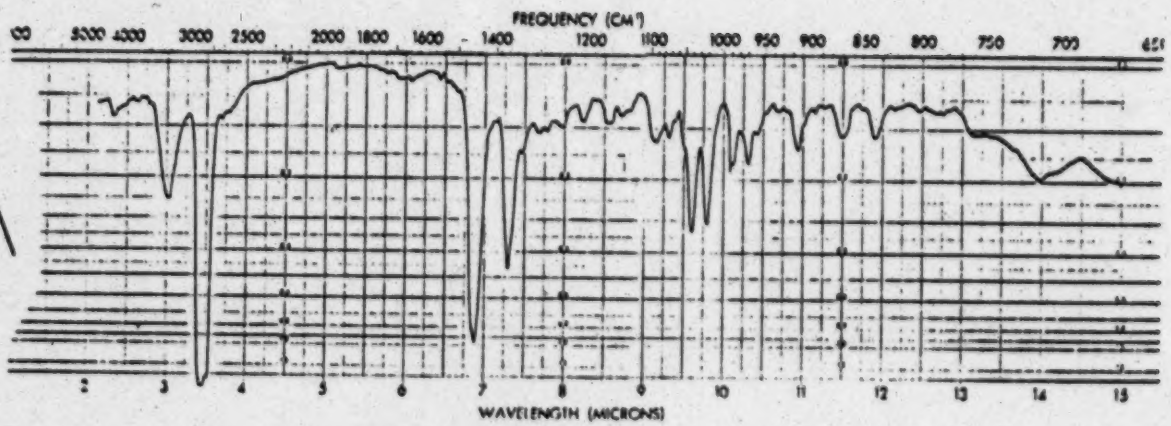

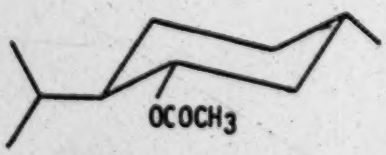

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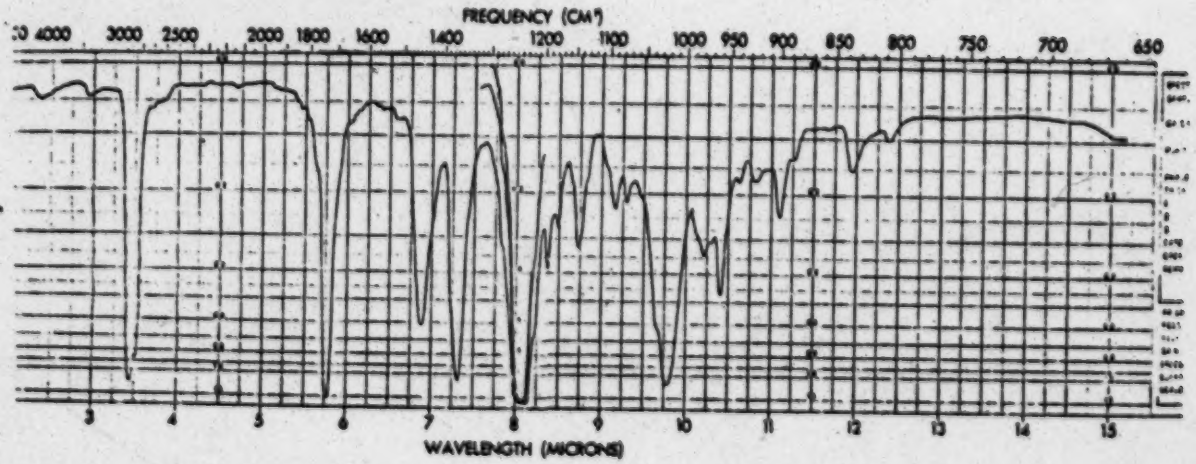
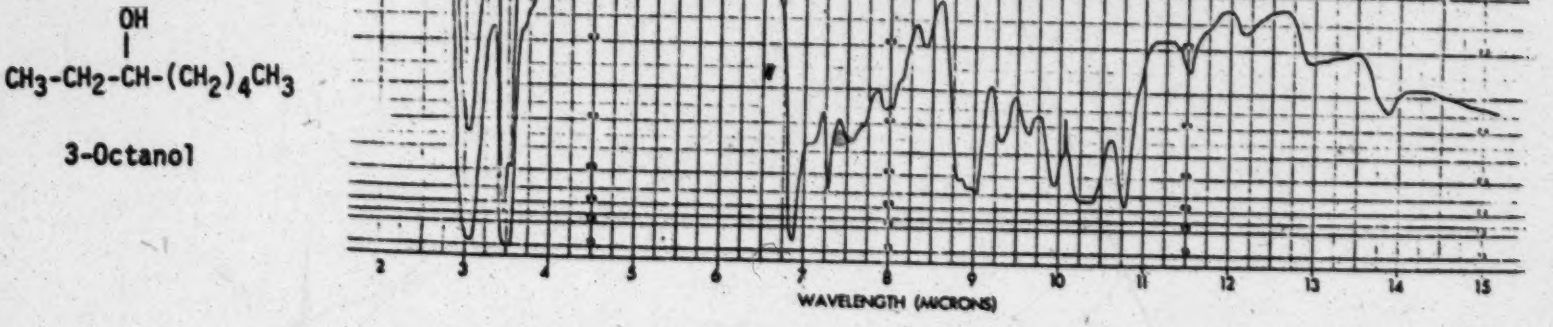
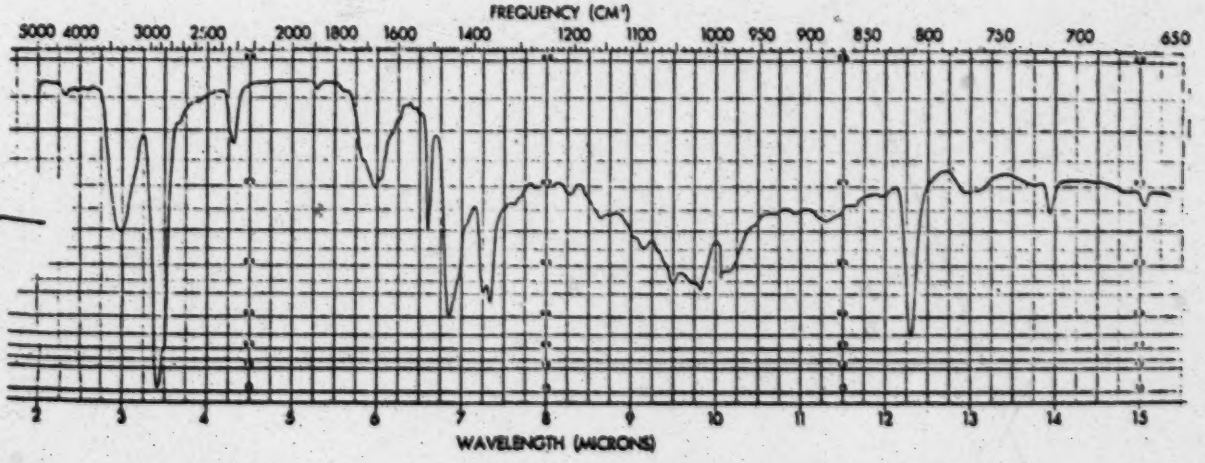


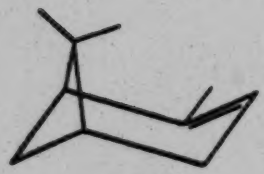


OH
|
$CH_3-CH_2-CH-(CH_2)_4CH_3$

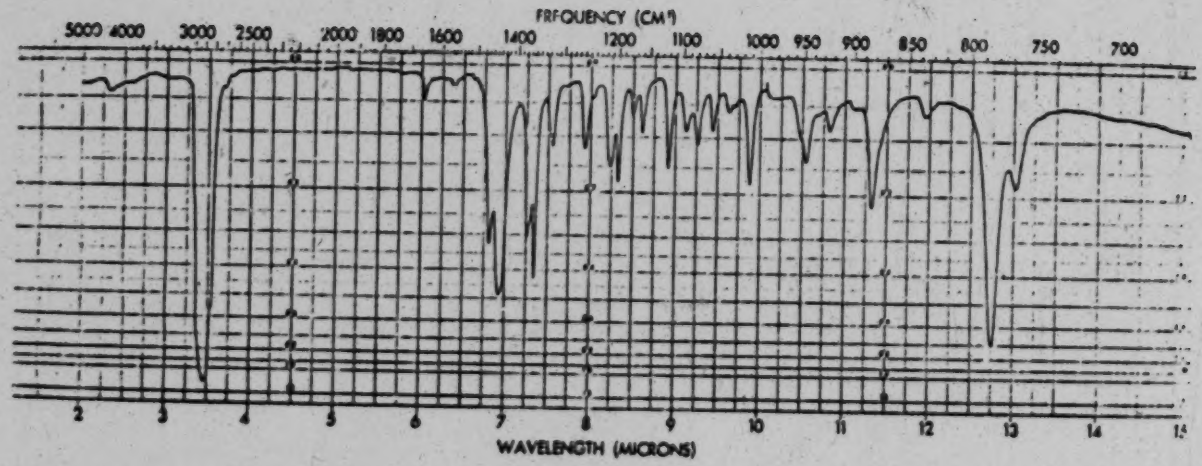
3-Octanol

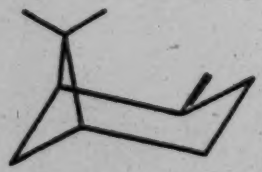


α-Phellandrene

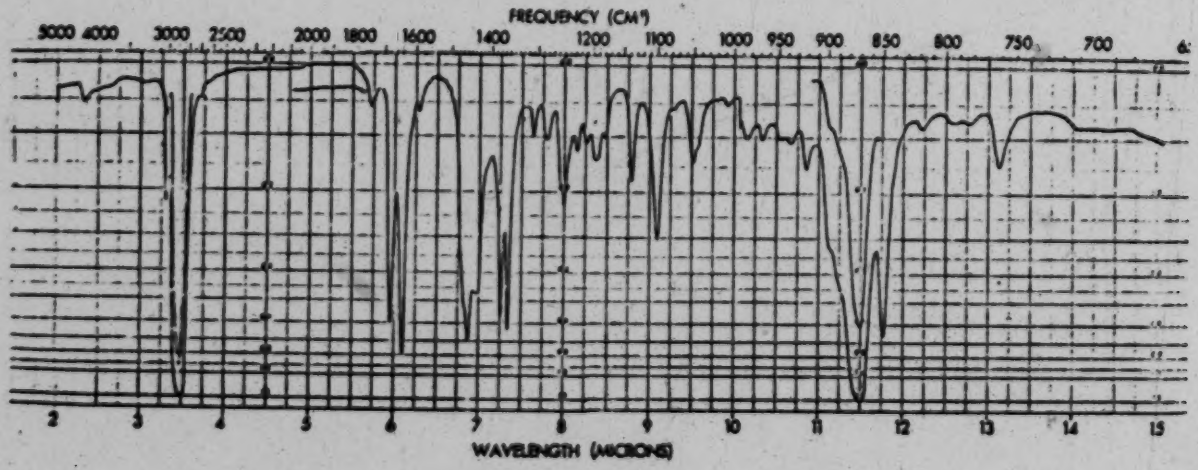


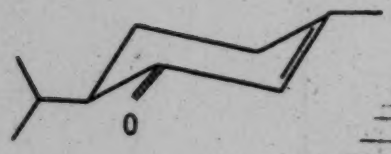
α-Pinene



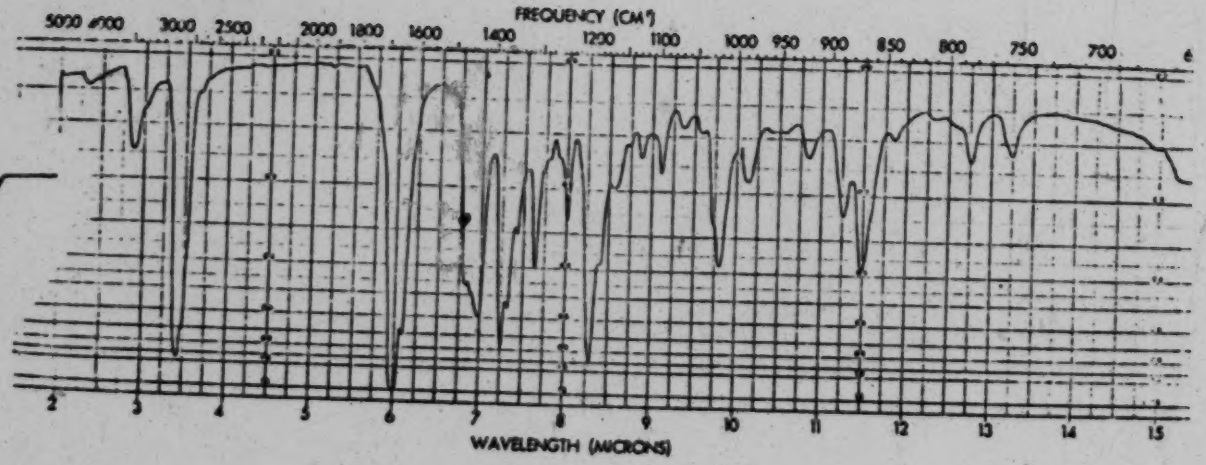


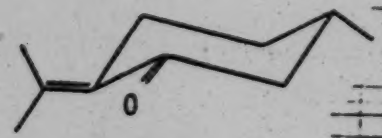
β-Pinene





Piperitone





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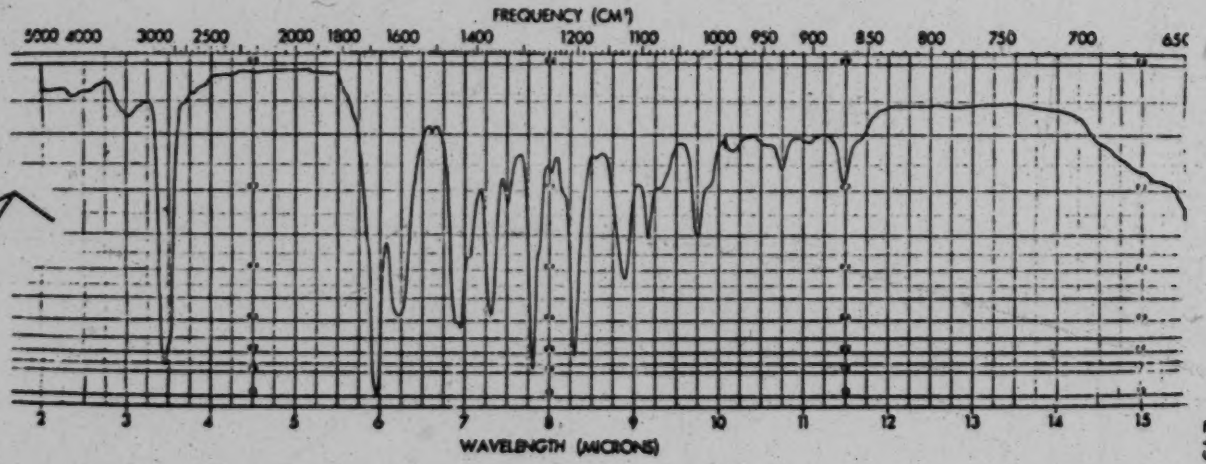
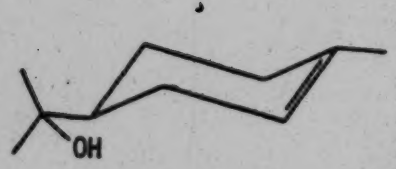
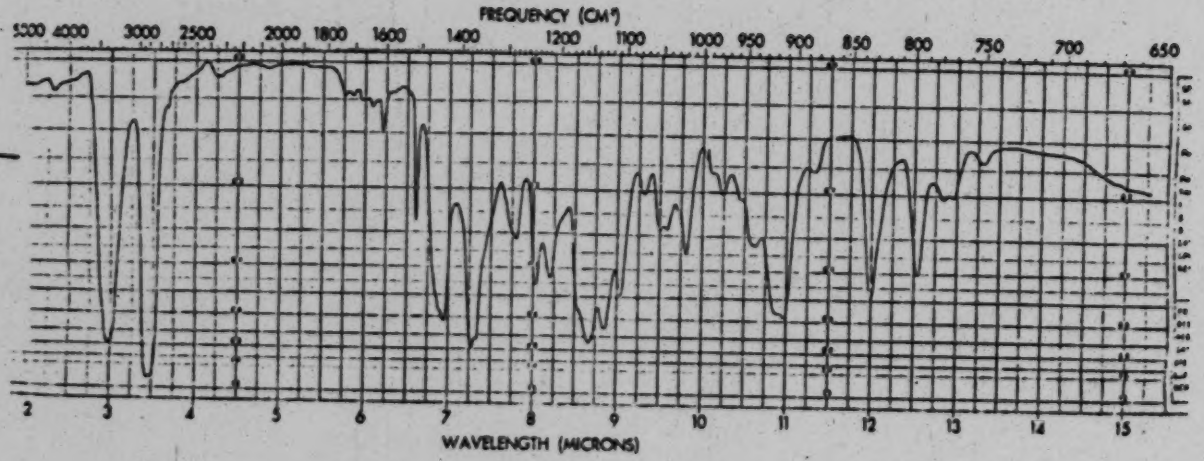
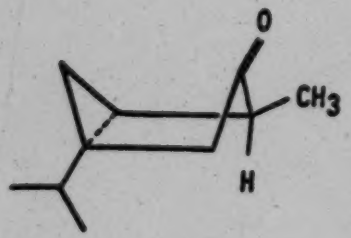


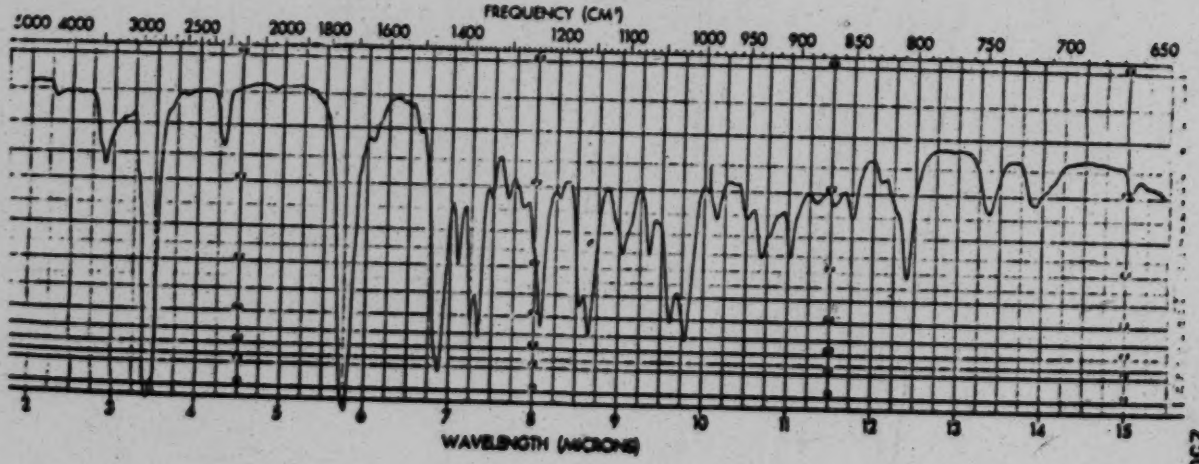


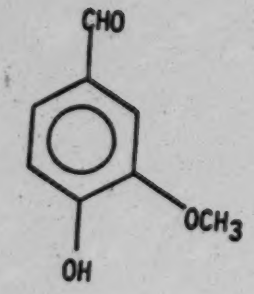
α-Terpineol



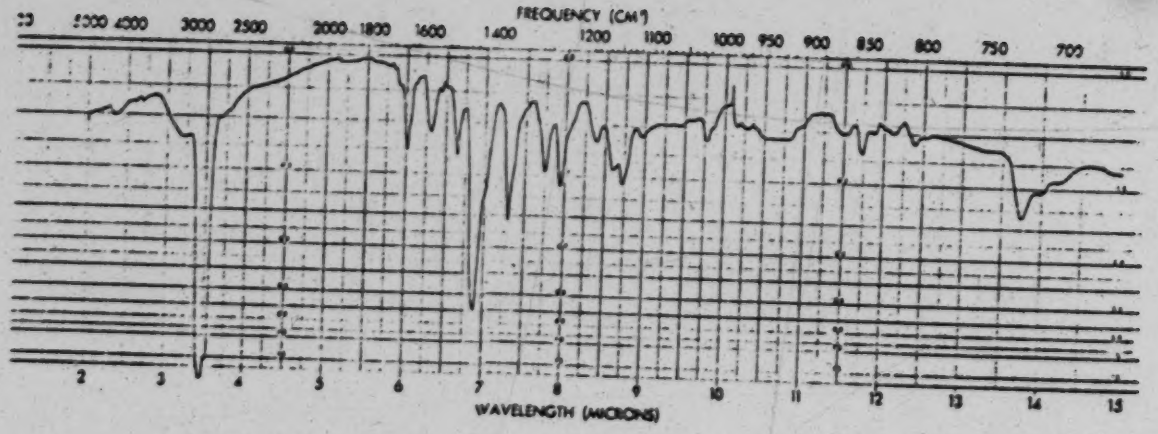


α-Thujone




CHO
OCH3
OH

Vanillin


FREQUENCY (CM-1)
5000 4000 3000 2500 2000 1800 1600 1400 1200 1100 1000 950 900 850 800 750 700
2 3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH (MICRONS)

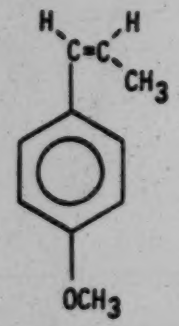
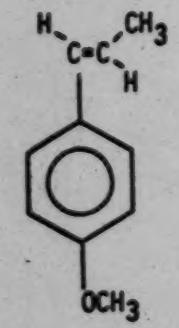
B-1

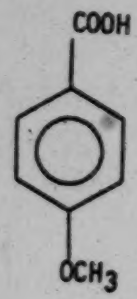
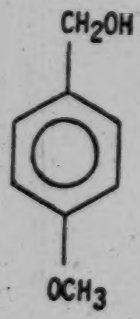
COMPOSITION OF PEPPERMINT OILS

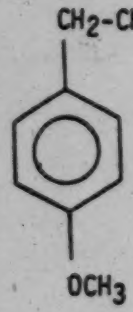
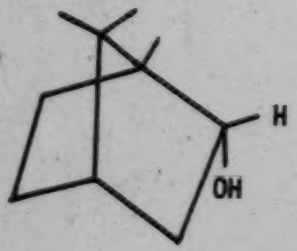
Sample, Provenance & Other Information Given by Supplier	Component %													
	-Pinene	Camphene	-Pinene	Limonene	Cineole	Menthofuran	Menthone	Isomenthone	Menthenol	Menthol	Menthyl acetate	Pulegone	Piperitone	Others
U.S. Midwest														
Natural	0.8	...	1.0	3.0	6.3	1.9	29.9	4.0	3.4	39.3	4.2	0.5	1.3	1.0
Natural	0.8	...	0.9	3.4	7.1	1.7	30.7	3.3	3.5	38.8	4.5	0.6	0.9	1.7
Natural	0.8	(0.2)	0.9	3.6	8.7	0.7	31.6	0.9	3.6	37.0	5.1	0.8	0.5	0.8
Midwest-Oregon blend, lightly rectified	0.5	...	0.8	2.7	7.3	2.6	27.5	4.8	3.9	41.7	6.8	1.5	1.4	0.7
Midwest-Oregon blend, heavily rectified	0.1	...	0.1	0.7	1.9	1.9	24.4	4.2	5.0	51.4	6.6	1.1	2.1	0.4
U.S. Oregon														
Natural	0.8	...	0.9	4.3	8.1	3.2	19.5	4.5	4.1	43.1	6.1	2.3	1.7	1.2
Natural	0.7	...	1.0	3.6	8.0	3.3	23.8	4.8	3.6	41.8	4.4	2.1	2.3	1.5
Natural, Jefferson district	0.6	(0.2)	1.6	3.2	7.5	2.6	21.3	3.2	4.2	46.2	4.5	0.9	3.1	1.1
U.S., Yakima														
Sunnyside, natural	0.5	(0.2)	1.0	4.3	8.1	0.8	16.7	3.5	3.6	43.2	6.9	1.9	0.7	0.8
Mammoth, natural, plants grown in Sunnyside area	0.8	(0.1)	1.1	3.7	7.0	0.1	17.1	3.4	3.3	42.2	7.4	2.2	1.0	1.0
Yakima, natural, early harvest	0.6	(0.2)	1.7	3.6	13.5	0.2	17.9	3.0	2.8	40.2	5.3	2.6	1.4	1.2
Yakima, natural, late harvest	0.7	...	1.6	3.7	6.4	0.4	8.9	2.2	3.3	48.7	11.6	0.9	1.1	0.9
Rectified U.S.P.	0.7	(0.3)	1.2	3.7	8.8	0.2	15.7	3.2	3.6	41.9	8.6	1.3	0.8	1.3
Terpeneless, produced from P-1 by vacuum fractionation	0.1	...	0.2	1.3	2.5	0.5	16.1	3.6	4.2	51.9	10.0	2.5	1.2	1.2
Italian														
Italo-Mitcham, rectified	0.7	(0.2)	0.9	4.2	8.0	0.3	21.0	3.6	3.5	48.2	5.3	3.3	1.7	1.2
Italo-Mitcham, rectified	0.2	(0.1)	1.0	4.6	7.7	0.4	16.8	5.1	3.9	35.2	6.1	4.6	2.9	3.6
Rectified	0.8	...	1.0	4.2	7.0	0.8	17.6	3.9	3.9	40.4	5.6	3.9	1.9	2.0
Italo-Mitcham, distilled	1.0	...	1.1	6.0	8.9	0.4	18.1	3.3	3.2	38.7	4.8	2.8	2.1	2.9
Italo-Mitcham, distilled U.S.P., A.P.	0.9	(0.3)	1.0	6.2	9.9	0.5	19.6	6.6	3.6	35.3	5.6	2.7	2.1	2.2
Terpeneless	0.9	(0.0)	0.2	2.3	1.3	4.3	21.0	4.3	3.7	49.4	5.7	4.3	1.3	1.7
Italo-Mitcham, distilled U.S.P.	0.7	(0.2)	1.6	3.9	7.5	0.1	19.8	4.9	3.5	46.0	6.5	3.5	2.7	1.1
Dutch														
Genuine, distilled	0.7	...	1.0	6.8	8.8	1.7	20.3	3.3	4.1	43.9	4.6	1.3	1.7	2.0
	0.7	...	1.0	4.0	9.4	1.6	21.0	3.3	4.1	46.7	3.8	0.8	1.6	2.0
Distilled U.S.P., A.P.	0.6	(0.3)	0.7	4.3	8.7	1.8	16.9	4.1	4.5	47.4	6.9	1.3	1.4	1.2
Mitcham, distilled	0.5	...	0.7	3.9	8.1	0.8	15.9	3.8	4.9	44.4	9.6	1.9	1.9	0.6
	0.5	(0.6)	1.3	5.5	7.1	0.8	15.6	5.9	5.3	39.7	8.5	2.2	2.0	1.0
Mitcham, distilled, 1950; dry & rainy season	0.8	(0.2)	1.0	4.0	12.4	4.5	18.2	4.5	3.6	38.6	4.6	2.7	4.1	5.6
Bulgarian														
Bulgaro-Mitcham, natural	0.7	...	1.6	3.5	7.5	6.2	16.9	3.1	3.8	43.9	7.2	2.7	1.9	1.1
Bulgaro-Mitcham, rectified	0.2	...	1.0	2.7	6.2	8.9	17.1	3.5	4.3	46.3	6.6	2.8	2.5	1.5
South African														
Natural, Mitcham-type "A", produced 1957	0.6	...	0.7	5.8	7.4	8.8	19.1	5.1	3.4	36.1	6.9	2.1	2.3	1.8
Natural, Mitcham-type "A", produced 1958	0.4	...	0.5	6.3	7.4	9.2	17.9	6.9	4.2	33.2	10.5	2.1	2.8	1.4
Argentine	0.6	(0.1)	1.5	3.8	6.7	8.4	12.8	2.7	3.2	46.9	7.5	2.1	2.2	1.7
Canadian														
Redistilled U.S.P., produced from plants grown in province of Ont.	0.8	(0.1)	1.9	3.9	8.9	1.2	26.5	4.1	4.2	40.6	1.9	0.6	1.7	1.6
Netherlands														
Experimental sample 1949 crop	0.5	(0.1)	1.3	1.0	4.4	0.3	17.8	4.2	3.6	54.5	6.5	0.9	3.5	1.0
Polish	0.6	(0.1)	1.7	3.9	7.9	3.1	24.8	4.2	3.8	46.1	4.5	0.9	2.1	2.5
Spanish	0.6	(0.1)	1.3	3.1	6.7	2.9	30.6	5.3	3.1	36.0	3.8	2.8	2.1	1.5

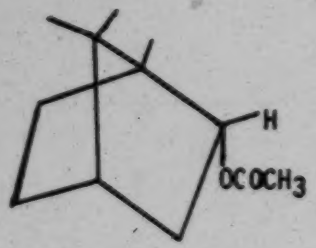
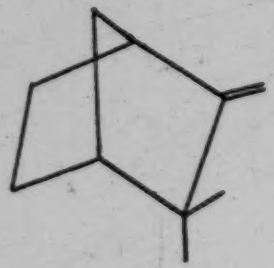
APPENDIX E: Mass Spectra Data

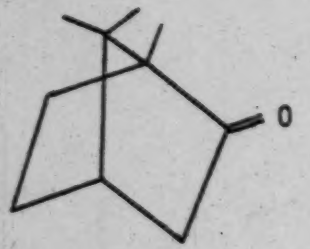
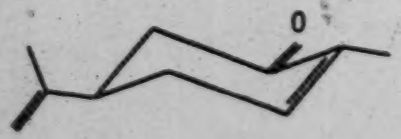
MASS SPECTRA DATA

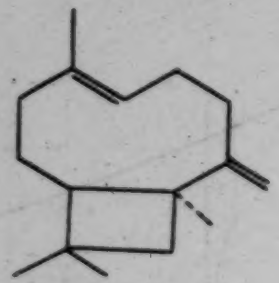
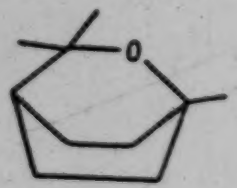
Component	Source	Peak Number of the Component in GLC	m/e
cis-Anethole 	anise oil	34	148 147 133 117 105 121 77 91
	fennel oil	45	148 147 117 44 105 133 91 57
	anise oil	34	148 147 133 117 105 121 91 115
trans-Anethole 	anise oil	38	148 147 117 133 77 121 105 91
	fennel oil	48	148 147 117 133 105 77 121 91
	standard		148 147 117 133 105 77 121 91
	Wiley		148 147 133 117 121 77 105 115
			148 147 117 105 77 133 91 79
	ALDN		148 147 117 105 77 133 91 79

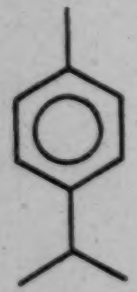
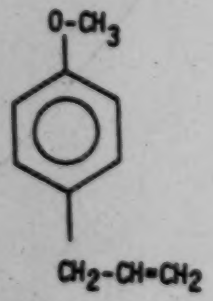
Component	Source	Peak Number of the Component in GLC	m/e
p-Anisic acid 	anise oil	44	162 131 161 104 103 77 135
	Wiley		132 152 77 107 92 63 136 84
			152 135 77 63 91 120 64 50
Anisyl alcohol 	anise oil	46	135 136 77 145 56 99 107 44
	standard		138 109 137 121 77 94 107 79

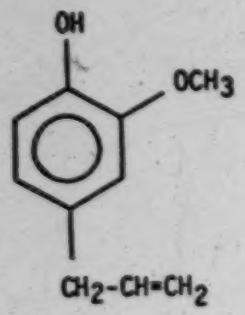
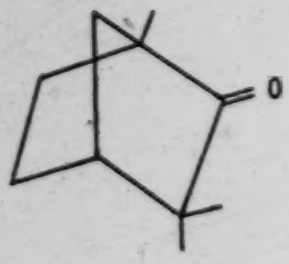
Component	Source	Peak Number of the Component in GLC	m/e
Anisyl acetone	standard		161 176 133 175 162 118 44 77
			
Borneol	sage oil	29	105 161 93 107 119 91 204 81
	standard		95 110 41 55 136 139 96 67
	Wiley		95 110 93 139 121 69 96 71
			95 95 121 81 91 79 41 67

Component	Source	Peak Number of the Component in GLC	m/e
Bornyl acetate	sage oil	27	95 43 136 93 131 41 80 107
	standard		95 43 136 121 93 41 108 196
	Wiley		43 93 41 121 55 80 67 92
Camphene	dill oil	2	93 119 91 92 77 134 44
	fennel oil	7	93 121 79 91 107 67 41
	peppermint oil	2	93 91 32 92 77 41 44 79
	sage oil	3	93 121 79 107 91 67 136
	Wiley		93 121 41 39 27 79 67 107
			93 121 79 41 107 67 91 77
			93 121 79 67 107 95 69 94

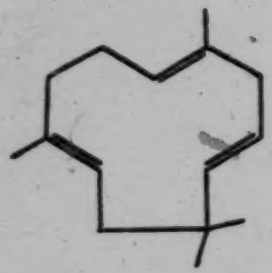
Component	Source	Peak Number of the Component in GLC	m/e
Camphor 	fennel oil	31	95 81 41 108 69 55 152
	sage oil	23	95 81 108 41 152 69 109 83
	standard		95 81 108 41 152 69 109 83
	Wiley		95 81 108 69 152 109 83 110
			126 95 41 81 39 69 108 55
Carvone 	anise oil	35	82 108 93 54 107 106 39 150
	anise oil	35	82 108 93 107 150 79 91 77
	caraway oil	42	82 54 108 93 39 107 41 106
	dill oil	31	82 108 54 93 39 107 41 106
	standard		82 64 44 108 135 54 93 107
	Wiley		82 54 108 93 58 107 106 39
			82 54 39 93 108 41 107 27

Component	Source	Peak Number of the Component in GLC	m/e
β-Caryophyllene 	anise oil	28	93 69 133 91 41 79 81 121
	caraway oil	36	93 41 69 91 44 121 79 105
	sage oil	28	93 69 41 133 81 79 85 91
	Wiley		93 41 69 133 91 79 105 107
1,8-Cineol 	dill oil	9	93 91 68 77 119 136 92 78
	fennel oil	14	68 93 67 79 94 136 78 77
	peppermint oil	12	68 93 136 67 121 81 79 43
	sage oil	13	93 68 136 77 121 79 94 91
	Wiley		43 28 71 81 84 108 69 55

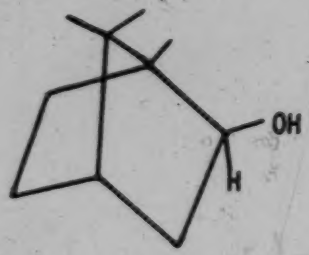
Component	Source	Peak Number of the Component in GLC	m/e
p-Cymene	dill oil	12	119 134 91 120 117 77 93 115
	peppermint oil	16	119 93 91 77 134 136 121 79
	sage oil	17	119 134 91 107 120 77 93 105
	Wiley		119 134 91 28 120 77 65 41
			119 134 91 28 120 117 41 78
Estragole	anise oil	29	148 93 119 69 121 147 107 161
	fennel oil	42	148 147 121 117 133 77 105 91

Component	Source	Peak Number of the Component in GLC	m/e
Eugenol	anise oil	51	107 145 56 99 149 44 136
	anise oil	51	164 57 149 145 56 165 99
	standard		164 149 55 77 131 103 137
	Wiley		164 15 27 76 148 38 90 54
			164 149 131 137 103 77 133 165
Fenchone	anise oil	14	81 69 41 152 80 82 109 67
	fennel oil	27	81 69 41 152 80 109 68 82
	Wiley		69 81 41 80 82 67 109 68

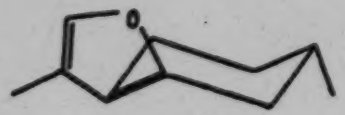
Component	Source	Peak Number of the Component in GLC	m/e
Humulene (α-Caryophyllene)	sage oil	33	93 121 80 147 204 107 92 41
	Wiley		93 80 121 41 92 147 79 107
			93 53 67 39 91 79 27 77
	ALDN		41 79 91 39 53 77 67 204

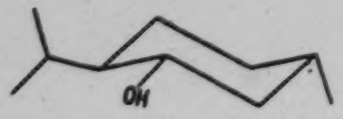


Isoborneol	standard		95 110 41 136 139 96 55 93
	Wiley		95 41 27 43 39 93 55 29
			95 41 43 110 93 55 136 69
			95 93 110 121 82 96 136 92



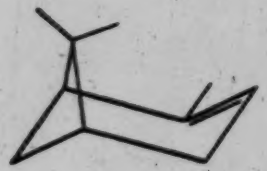
Component	Source	Peak Number of the Component in GLC	m/e
Isomenthone	peppermint oil	34	112 69 139 154 41 55 111
	Wiley		112 69 41 55 43 139 70 56
Limonene	anise oil	6	68 93 67 94 136 77 121 107
	caraway oil	11	68 93 67 94 136 79 121 92
	dill oil	8	93 68 67 79 136 41 52 39
	fennel oil	13	68 93 67 136 94 121 79 107
	peppermint oil	10	68 93 67 94 136 79 121 92
	sage oil	11	68 93 67 79 94 136 121 92
	Wiley		68 93 67 39 41 27 53 79
			68 67 93 94 79 92 121 107
			68 93 67 79 136 94 121 41

Component	Source	Peak Number of the Component in GLC	m/e
Linalool	standard		93 121 136 41 68 91 56 79
	Wiley		93 71 41 55 43 69 80 67
			71 43 41 93 55 69 80 67
			71 93 41 43 69 55 80 39
Menthofuran 	peppermint oil	28	108 150 79 109 77 91 151 80
	standard		108 150 56 79 44 145 99 122
	Wiley		108 150 39 79 27 77 41 109
			108 150 79 109 39 77 41 91

Component	Source	Peak Number of the Component in GLC	m/e
Menthol 	peppermint oil	42	95 81 71 55 82 67 41 96 123
	standard		71 81 95 138 82 123 55 41 96
	Wiley		71 81 95 55 82 138 41 69
			71 81 95 82 123 96 69 67
Menthone	peppermint oil	31	112 69 41 55 139 154 70 56
	Wiley		112 69 139 111 154 97 70 55
			112 69 139 111 154 70 55 54

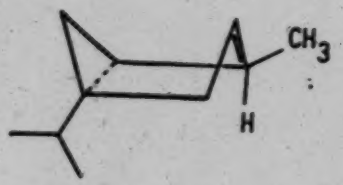
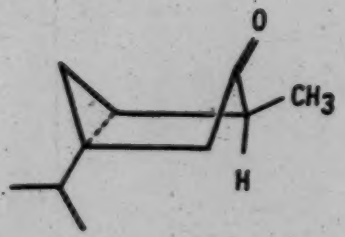
Component	Source	Peak Number of the Component in GLC	m/e
Menthyl acetate	peppermint oil	39	95 81 138 123 67 69 82 93
	standard		95 81 67 152 80 44 82 109
	Wiley		43 95 138 81 41 39 55 262
Myrcene	caraway oil	10	93 69 41 91 77 39 94 92
	dill oil	7	93 69 41 121 91 136 68 77
	fennel oil	12	93 41 69 91 79 77 39 67
	peppermint oil	9	93 41 69 91 77 79 92 136
	sage oil	10	93 69 41 91 79 77 39 121
	Wiley		41 93 69 39 27 53 79 67
			93 41 69 79 77 53 91 67
			41 69 93 79 67 77 53 136
			41 69 93 97 67 91 77 53

Component	Source	Peak Number of the Component in GLC	m/e
3-Octanol	peppermint oil	24	55 59 70 41 83 85 57 43
	Wiley		59 55 83 41 29 31 27 43
			59 29 43 55 27 41 31 83
α-Phellandrene	anise oil	4	93 91 136 77 92 121 119
	anise oil	4	93 119 91 77 92 136 134
	caraway oil	9	93 91 77 136 79 119 92
	dill oil	6	93 91 77 92 119 136 121
	fennel oil	11	93 91 77 79 136 41 94
	peppermint oil	8	93 91 77 79 136 41 92
	sage oil	8	93 91 77 79 136 121 41
	Wiley		93 91 77 92 136 39 27 41
			93 91 77 92 136 43 94 79

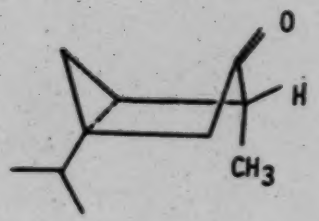
Component	Source	Peak Number of the Component in GLC	m/e
α-Pinene 	caraway oil	2	93 92 91 77 79 121 136
	dill oil	1	93 92 91 77 79 121 136
	fennel oil	4	93 121 91 92 77 79 68
	peppermint oil	1	93 92 91 77 79 121 41
	sage oil	3	93 91 92 121 77 79 136
	standard		93 121 68 91 136 79 77
	Wiley		93 92 91 77 79 41 95 136
			93 92 91 77 79 41 121 94
			93 92 91 77 79 121 94 80
β-Pinene	caraway oil	5	93 69 91 41 79 77 92 121
	dill oil	3	93 91 119 77 79 136 44 92
	fennel oil	8	93 41 69 91 79 77 92 94
	peppermint oil	4	93 41 69 91 79 77 94 121
	sage oil	6	93 69 41 79 77 121 94 136
	standard		93 119 44 41 91 121 69
	Wiley		93 41 69 79 77 91 94 121
			93 41 69 79 91 71 94 80
			119 134 91 28 120 117 41 136

Component	Source	Peak Number of the Component in GLC	m/e
Piperitone	peppermint oil	54	82 110 95 137 109 41 152 54
	Wiley		82 95 137 54 109 152 41 110

Pulegone	peppermint oil	47	81 152 67 109 161 93 41 82
	Wiley		81 67 152 109 43 82 69 42

Component	Source	Peak Number of the Component in GLC	m/e
α-Thujene 	sage oil	1	93 136 91 92 121 79 77 41
	Wiley		93 77 91 92 27 41 39 79
			93 91 77 92 41 79 136 94
α-Thujone 	sage oil	21	119 41 81 110 68 39 69 67 95

Component	Source	Peak Number of the Component in GLC	m/e
β-Thujone	sage oil	22	119 134 110 91 81 117 132 109
Thujone	Wiley		81 68 67 69 95 41 109 55



Wiley: Atlas of Mass Spectral Data, John Wiley & Son, N.Y.
ALDN: Aldermaston 8 peak Index of Mass Spectra, ICI

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